R E M A R K S

The Office Action dated January 15, 2003 presents the examination of claims 6, 7, 9, 12, 16, and 17. Claims 6 and 7 are amended. The amendment clarifies that the tumor antigen protein of claims 6 or the tumor antigen peptide of claim 7 binds to MHC class I antigen and is recognized by tumor-specific CTLs, after intracellular decomposition or as it is. Support for this amendment is found in the specification, such as on page 13, line 7 and page 24, line 10. Claims 16 and 17 are canceled. No new matter is inserted into the application.

Maintained Rejection under 35 U.S.C. § 112, first paragraph

The Examiner rejects claims 16 and 17 under 35 U.S.C. § 112, first paragraph for allegedly not being described by the specification. Claims 16 and 17 are canceled, thus rendering the rejection moot.

New Rejection under 35 U.S.C. § 101

The Examiner rejects claims 6, 7, 9, 12, 16, and 17 under 35 U.S.C. § 101 for allegedly not being supported by either a credible, substantial or well-established utility. Claims 16 and 17 are canceled, thus rendering the rejection of these claims moot.

Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

On page 5, lines 8-10 of the outstanding Office Action, the Examiner asserts,

"[T]here is no objective evidence, either in the specification or in the prior art of record, which discloses or suggests that SEQ ID NO:2 plays a role in cancer, autoimmune disorders, or is a tumor antigen peptide."

Applicants strongly disagree with the Examiner's position. The protein of the present invention is an established tumor antigen protein of which utility is described in the specification and is known is the art, as explained below.

First, at the priority date, it was known in the art that a "tumor antigen" means a target of T cells including cytotoxic T cells (CTLs) that recognize tumor cells, i.e., tumor-specific T-cells, as shown in the prior art references disclosed in the instant specification and in van der Bruggen et al. *Science*, 254:1643 (1991), attached hereto as **Exhibit 1**. Specifically, Example 3 and especially Table 2 on pages 32-35 of the instant specification show that KE-4CTL produces IFN-γ in response to cells that have been co-transfected with a nucleotide sequence encoding the tumor antigen

protein of the present invention (plasmid K3) and a nucleotide sequence encoding HLA antigen. KE-4CTL is known in the art to be a "tumor-specific CTL" that recognizes and kills squamous cell carcinoma cell line KE-4 derived from esophageal cancer patients (see, Reference Example 1, pages 26-27 of the specification). Further, as demonstrated in Example 3, said "tumor-specific CTL" recognizes the protein of the present invention, and is subsequently induced and activated. This data presented in the specification clearly demonstrates that the protein of the present invention is truly a "tumor antigen protein."

Second, the protein of the present invention is well accepted in the art as a "tumor antigen protein." See, for example, Renkvist et al., *Cancer Immunol Immunother*, 50:3-15 (2001), attached hereto as **Exhibit 2**. This publication by a third party not associated with the present inventors, entitled "A listing of human tumor antigens recognized by T cells," lists SART-1 of the present invention as one of the tumor antigens in Table 3: "Class I HLA-restricted widely expressed antigens" (see, pages 6-7). In another publication by a third party not associated with the present inventors, Rosenberg et al., *Immunity*, 10:281-287 (1999), attached hereto as **Exhibit 3**, SART-1 is listed as one of tumor antigens at page 282, Table 1: "Defined Cancer Antigens." Exhibits 2 and 3 serve as objective

evidence that SART-1[1] of the present invention is, in fact, a "tumor antigen protein."

Third, the specific utility of SART-1, the protein of the present invention, is described in detail at page 24, line 1 to page 25, line 1 of the specification. In this respect, clinical trials have recently been carried out using a peptide derived from SART-1 of the present invention, and are published in Yamana, H. and K. Itoh, *Cancer and Chemotherapy*, 27(10):1477-1488, attached hereto as **Exhibit 4**. SART-1 is listed on page 1479 of Exhibit 4. In the English summary at page 1477, the authors disclose that they are performing phase I trials using SART-1-derived peptide vaccines and that they have found an increase in CTL precursor frequency in some cases in an *in vitro* study. These facts are consistent with the guidance provided in the instant specification and demonstrate that SART-1 of the present invention is actually a "tumor antigen protein."

Finally, the Examiner relies on Kast et al. in asserting the following on page 5, last line to page 6, line 2 of the outstanding Office Action:

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[1] The fact that the tumor antigen protein of the present invention is referred to as "SART-1" in the art has already been set forth in the Declaration under 37 C.F.R. § 1.132 filed with the United States Patent and Trademark Office on June 8, 2001.

"Thus just because there is a correlation of the claimed peptides and this observation does not result in the proteins being tumor antigens."

Amended claims 6 and 7 recite that CTLs that recognize the decomposition products of the protein of claim 6 or the peptides of claim 7 are tumor-specific. Therefore, the protein or peptide of the present invention is a "tumor antigen" and distinct from a "virus antigen." The fact that the protein (as recited in claim 6) and the peptide (as recited in claim 7) of the present invention are recognized by tumor-specific CTLs is shown in Example 3 (Table 2) and Examples 4-5 (Tables 3, 4, 5, and 6), respectively, of the specification.

Based upon the above objective evidence and description in the specification, it is clear that the protein of SEQ ID NO:2, as recited in the claims, has specific and practical utility. The rejection under 35 U.S.C. § 101 is therefore improper and should be withdrawn.

Rejection under 35 U.S.C. § 112, first paragraph, enablement

The Examiner rejects claims 6, 7, 9, 12, 16, and 17 under 35 U.S.C. § 112, first paragraph for an alleged lack of enablement. Claims 16 and 17 are canceled, thus rendering the rejection of these

claims moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner relies on Boon et al. and Kast et al. to assert the following:

"Thus just because there is a correlation of the claimed peptides and this observation does not result in the proteins being tumor antigens. Therefore, in view of the lack of guidance in the specification and in view of the unpredictability in the art as evidenced from Boon et al and Kast et al in view ... one of skilled in the art would be required to perform undue experimentation in order to practice the claimed invention." See, page 8, lines 2-7, of the outstanding Office Action.

Applicants respectfully disagree with the Examiner's position for the following reasons.

First, as described above, a SART-1-derived peptide vaccine has recently been used as a peptide vaccine in clinical trials, which means that the protein/peptide of the present invention is practically useful as described in the present specification.

Second, determination of enablement is to be weighing of several factors (Ex Parte Forman, 230 USPQ 546 (BPAI 1986)), as the Examiner stated, and the factor of "undue experimentation" is not

related to the quantity of experimentation but whether undue experimentation is needed to practice the full scope of the invention (In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988)). In this regard, the present invention can be practiced by the skilled artisan without "undue experimentation."

The Examiner seems to rely heavily upon the unpredictability factor, making reference to Boon et al. or Kast et al. These references, however, are irrelevant to the present application. Instead, the issue of predictability in the instant case should be decided on whether it is predictable that one of skilled in the art, given one functional embodiment of the present invention, can find another. In this regard, the instant specification provides sufficient description so as to enable one of ordinary skill in the art to carry out the present invention without undue experimentation. That is, (a) the present invention is directed to a protein or a peptide, which is constrained by *i)* amino acid or nucleotide sequence, and *ii)* activity of binding to MHC class I antigen and being recognized by tumor-specific CTLs after intracellular decomposition or as it is; (b) the skill of the practitioner in the art to which the present invention pertains is very high; (c) the specification provides considerable guidance for determining whether a candidate protein or peptide has the claimed

activity, and determination thereof can be performed using methods described in the specification or other methods known in the art and available to a skilled person at the priority date; (d) the specification provides Examples of the preparation of protein and peptide of the present invention and further provides Examples of an assay that can be performed to determine if any particular embodiment possesses the activity of binding to HLA and being recognized by tumor-specific CTLs; (e) the quantity of experimentation required to screen protein/peptide is not unexpectedly large.

Based upon the above, it is clear that subject matter recited in the claims is enabled by the specification. The rejection under 35 U.S.C. § 112, first paragraph, is therefore improper and should be withdrawn.

Summary

All of the present claims define patentable subject matter such that this application should be placed into condition for allowance. The Examiner is respectfully requested to issue a Notice of Allowability indicating that claims 6, 7, 9, and 12 are allowed.

If there are any issues remaining that may be solved through a telephone conversation, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at 703-205-8000.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicants hereby petition for an extension of two (2) months to June 15, 2003, in which to file a reply to the Office Action. The required fee of $410.00 is enclosed herewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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By Kristi L Rupert #45,702
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Attachment: Version with Markings to Show Changes Made
Exhibit 1 - P. Van Der Bruggen et al., Science
Exhibit 2 - N. Renkvist et al., Cancer Immunol. Immunother.
Exhibit 3 - S. Rosenberg et al., Immunity
Exhibit 4 - H. Yamana et al., Jpn J. Cancer Chemother.

Version with Markings to Show Changes Made

IN THE CLAIMS:

Claims 16 and 17 are canceled.

The claims have been amended as follows:

6. (Five Times Amended) An isolated tumor antigen protein selected from the group consisting of:

(a) a protein comprising an amino acid sequence shown in SEQ ID NO:2; and

(b) a protein encoded by a DNA comprising a nucleotide sequence shown in SEQ ID NO:1,

wherein said protein yields, through intracellular decomposition, peptide fragment(s) which binds to major histocompatibility complex (MHC) class I antigen and is recognized by tumor-specific cytotoxic T lymphocytes (CTLs) in such binding state.

7. (Four Times Amended) An isolated and chemically synthesized tumor antigen peptide that is a peptide fragment of a tumor antigen protein selected from the group consisting of:

(a) a protein comprising an amino acid sequence shown in SEQ ID NO:2; and

(b) a protein encoded by a DNA comprising a nucleotide sequence shown in SEQ ID NO:1,

wherein said tumor antigen peptide comprises the amino acid sequence of positions 749-757, 736-744, 785-793, or 690-698 in the amino acid sequence of SEQ ID NO:2, and binds to MHC class I antigen and is recognized by tumor-specific CTLs when bound to MHC class I antigen.

A Gene Encoding an Antigen Recognized by Cytolytic T Lymphocytes on a Human Melanoma

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Many human melanoma tumors express antigens that are recognized in vitro by cytolytic T lymphocytes (CTLs) derived from the tumor-bearing patient. A gene was identified that directed the expression of antigen MZ2-E on a human melanoma cell line. This gene shows no similarity to known sequences and belongs to a family of at least three genes. It is expressed by the original melanoma cells, other melanoma cell lines, and by some tumor cells of other histological types. No expression was observed in a panel of normal tissues. Antigen MZ2-E appears to be presented by HLA-A1; anti-MZ2-E CTLs of the original patient recognized two melanoma cell lines of other HLA-A1 patients that expressed the gene. Thus, precisely targeted immunotherapy directed against antigen MZ2-E could be provided to individuals identified by HLA typing and analysis of the RNA of a small tumor sample.

MOST MOUSE TUMORS EXPRESS ANtigens that constitute potential targets for rejection responses in syngeneic hosts (*1*). Against some of these tumors, highly active and specific CTLs can be derived from immunized animals by restimulation in vitro with tumor cells (*2*). That the antigens recognized by these CTLs in vitro can be effective tumor-rejection antigens is indicated by the finding that tumor cells that had escaped immune rejection in vivo were found to be resistant to the tumor-specific CTLs (*3*).

For human tumors, autologous mixed cultures of tumor cells and lymphocytes can generate CTLs that lyse the tumor cells (*4*). These anti-tumor CTLs do not lyse targets of natural killer cells and autologous control cells such as fibroblasts or EBV-transformed B lymphocytes. However, it is difficult to evaluate to what extent the antigens recognized on human tumors by autologous CTLs are relevant for tumor rejection.

We have obtained a panel of autologous CTL clones (*5*) that lyse melanoma cell line MZ2-MEL, which was derived from patient MZ2. By selecting clonal sublines of MZ2-MEL that are not killed by these CTL clones, we obtained antigen-loss variants that were resistant to subsets of the CTL clones, demonstrating that autologous CTLs recognize a total of six independent antigens on MZ2-MEL (*6*). We then attempted to identify the gene for one of these antigens, MZ2-E, by an approach that had enabled the isolation of genes for antigens recognized by CTLs on mouse tumors (*7*). This approach is based on the transfection of cosmid libraries prepared with DNA of cells that express the relevant antigen. Transfectants expressing the antigen are identified by their ability to stimulate the appropriate CTLs. As a first step, we tried to obtain transfectants expressing antigen MZ2-E (E) with genomic DNA of an E^+ MZ2-MEL subline. The DNA was transfected into E^- antigen-loss variant MZ2-MEL.2.2, which had been obtained by selection with an anti-E CTL clone. A transfectant was obtained that was lysed by autologous anti-E CTL clone 82/30 (*8*).

A library of 700,000 independent cosmids was prepared with DNA of an E^+ MZ2-MEL subline and groups of 50,000 cosmids were amplified (*9*). DNA from each group of cosmids was cotransfected into E^- line MZ2-MEL.2.2 together with plasmid pSVtkneoβ, that confers resistance to geneticin (*10*). Approximately 5000 geneticin-resistant transfectants were obtained for each group and they were divided into microcultures of 30 independent transfectants. These microcultures were allowed to expand, duplicated, and tested for their ability to stimulate tumor necrosis factor (TNF) release by anti-E CTL clone 82/30 (*11*). This enables identification of those pools that contain one transfectant that expresses antigen E (*8*). Two of the 14 groups of cosmids produced positive microcultures, and the E^+ transfectants were recovered from the duplicates. After additional transfection experiments, a total of five E^+ transfectants out of 29,000 independent geneticin-resistant transfectants were obtained with the first positive group of cosmids and two out of 13,000 with the second group.

By packaging the DNA of cosmid transfectants directly into lambda phage components, it is sometimes possible to retrieve cosmids that contain the sequences of interest (*7, 12*). This procedure was unsuccessful here, so we rescued the transfected sequence by ligating DNA of the transfectant to appropriate restriction fragments of cosmid vector pTL6 (*13*). This was tried with two transfectants and was successful with transfectant 7.4: cosmid B3 was obtained, which transferred the expression of antigen E at high efficiency. Transfectants obtained with this cosmid were as sensitive to lysis by the anti-E CTLs as the original melanoma cell line (Fig. 1).

Cosmid B3 was digested with Sma I and a 12-kb fragment could transfer the expression of the antigen (Fig. 1). After the fragment was digested with Bam HI, a 2.4-kb fragment was found to transfer the expression of antigen E at high efficiency (Fig. 1). This small fragment was used as a hybridization probe on a Southern (DNA) blot prepared with Bam HI–digested DNA of an

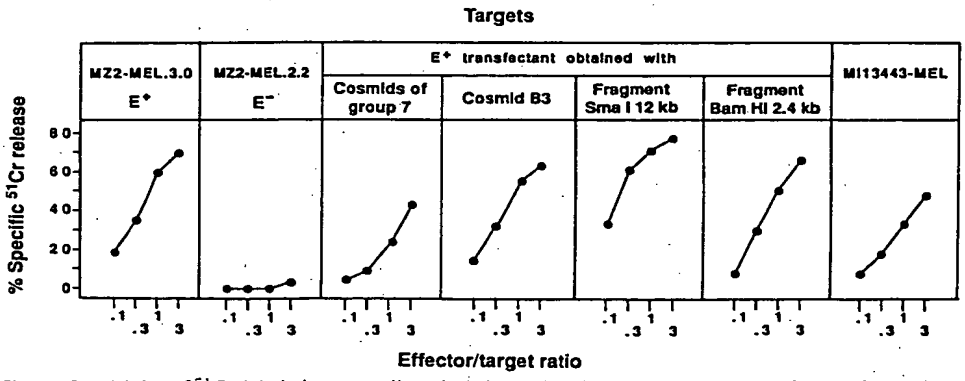

Fig. 1. Sensitivity of ^{51}Cr-labeled target cells to lysis by anti-E CTL clone MZ2-CTL 82/30. Chromium release was measured after 4 hours (*5*). MZ2-MEL.3.0 is a clonal subline of the melanoma cell line of patient MZ2, and MZ2-MEL.2.2 is an antigen-loss variant selected with an anti-E CTL clone (*6*). The E^+ transfectant clones were isolated from MEL.2.2 cell populations transfected with cosmid group 7 (transfectant 7.4); with cosmid B3; with a cloned 12-kb Sma I fragment of B3 (*20*); with a cloned 2.4-kb Bam HI fragment of the 12-kb fragment (*20*). Melanoma cell line MI13443-MEL was derived from another HLA-A1 patient.

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E^+ subline of MZ2-MEL and of the E^- antigen-loss variant. The expected 2.4-kb band was observed only with the DNA of the E^+ melanoma cell (Fig. 2), indicating that the E^- variant had lost the expression of the antigen as a result of a deletion affecting the relevant gene. Partial or complete deletions of tumor-antigen genes also occur in antigen-loss variants of mouse tumor cells (*14*). In addition to the 2.4-kb band, the probe hybridized to several additional bands of different intensities, suggesting that the gene responsible for the production of antigen E has sequence similarities to several other genes.

The sequence of the transfecting 2.4-kb genomic segment showed no significant similarity to any sequence presently recorded in data banks (*15*). Northern (RNA) blots and a cDNA library were prepared with RNA of E^+ subline MZ2-MEL.3.0 (*16*). The 2.4-kb segment hybridized to an mRNA of approximately 1.8 kb on a Northern blot. cDNA clones were obtained whose sequences were identical to parts of the 2.4-kb genomic fragment, thereby identifying two exons in this fragment. The position of one additional exon located upstream was obtained by sequencing segments of cosmid B3 that were located in front of the 2.4-kb Bam HI fragment. The gene extends over approximately 4.5 kb (Fig. 3). The starting point of the transcribed region was confirmed by polymerase chain reaction (PCR) amplification of the 5′ end of the cDNA (*17*). The three exons are 65, 73, and 1551 bp, respectively (Fig. 3). An ATG located in position 66 of exon 3 is followed by an open reading frame of 828 bp.

The ability of the 2.4-kb gene fragment to transfer the expression of antigen E confirms

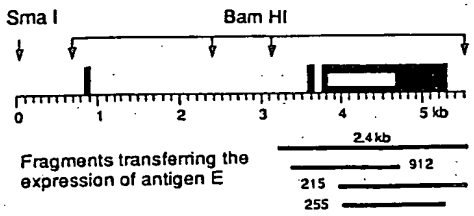

Fig. 3. Structure of the gene of antigen MZ2-E and restriction sites. Exons are indicated as black boxes and the open reading frame in exon 3 is marked in white. Boundaries of transfecting fragments are indicated relative to the first nucleotide of exon 3.

previous observations that truncated genes lacking the promoter can efficiently transfer CTL epitopes (*7*). Smaller regions of the gene corresponding to parts of the 2.4-kb fragment were cloned and tested by transfection into E^- cells. Three segments transferred the expression of the antigen (Fig. 3). Thus, the gene probably encodes the antigenic peptide recognized by the anti-E CTLs, as opposed to producing a protein that activates the encoding gene. The encoding sequence would be where all transfecting fragments overlap, as is the case for the four mouse tumor antigens that we have studied.

Two nonidentical cDNA species were also found when the cDNA library was probed with the 2.4-kb fragment. This confirmed the existence of a gene family suggested by the pattern observed on the Southern blots (Fig. 2). In contrast with the first cDNA, the second and the third types of cDNA were unable to transfer the expression of antigen E in transfection experiments. No significant homology was found by comparing the sequences of the two additional cDNAs to those presently recorded in gene banks (*15*). We propose the name *"MAGE"* (melanoma antigen) for this new gene family, with *MAGE-1* referring to the gene that directs the expression of antigen MZ2-E and *MAGE-2* and *-3* for the two other genes (Fig. 4). Analysis of the third exon showed that the two additional cDNAs are more closely related to each other (12% differences) than to the first (18.1% and 18.9% differences). Out of nine cDNA clones obtained with RNA of MZ2-MEL.3.0, three of each type were obtained, suggesting approximately equal expression of the three genes. It is possible that other closely related genes are expressed in lower amounts in these cells.

Experiments with mouse tumors showed that new antigens recognized by T cells can result from point mutations in the coding region of active genes. New antigens can also arise from the activation of genes that are not expressed in most normal cells (*7*). To clarify this issue for antigen MZ2-E, we compared the *MAGE-1* gene present in the melanoma cells to that present in normal cells of patient MZ2. We amplified by polymerase chain reaction (PCR) the DNA of phytohemagglutinin-activated blood lymphocytes with primers surrounding a 1300-bp stretch covering the first half of the 2.4-kb fragment. A PCR product was obtained, whereas none was obtained with the DNA of the E^- variant. The sequence of this PCR product was identical to the corresponding sequence of the gene carried by the E^+ melanoma cells. Moreover, we found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. Thus, the activation of a normal gene is responsible for the appearance of antigen MZ2-E. One may wonder how E^- antigen-loss variants could be obtained in these circumstances, because both copies of the gene would have to be inactivated. The same phenomenon has been observed with homozygous mouse tumor P815 (*3*). One explanation would be the existence among the

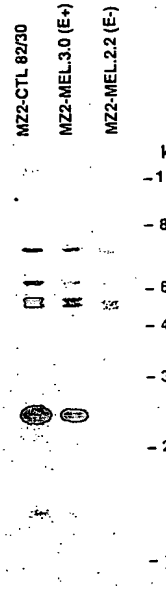

Fig. 2. Identification of a genomic deletion in the E^- antigen-loss variant. The 2.4-kb Bam HI fragment, which transferred the expression of antigen MZ2-E, was labeled with ^{32}P and used as a probe on a Southern blot of Bam HI–digested DNA of E^+ clonal subline MZ2-MEL3.0 and of E^- variant MZ2-MEL.2.2. The 2.4-kb band is absent in the lane of the E^- variant. The DNA of CTL clone 82/30 of patient MZ2 displayed the same bands as the E^+ melanoma cells.

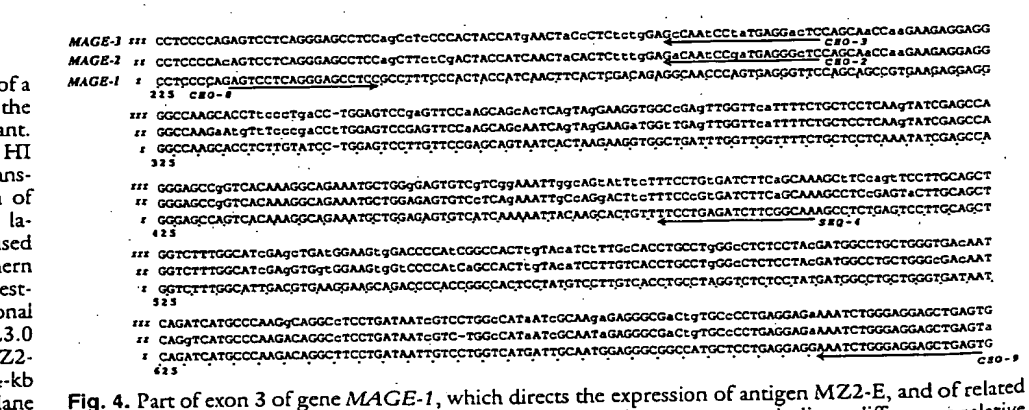

Fig. 4. Part of exon 3 of gene *MAGE-1*, which directs the expression of antigen MZ2-E, and of related genes *MAGE-2* and *MAGE-3*. Lowercase letters in the two latter sequences indicate differences relative to *MAGE-1*. Numbering is relative to the first nucleotide of exon 3. Oligonucleotides CHO-8 and CHO-9 were used to prime the reverse transcriptions and PCR amplification of Fig. 6. Oligonucleotides SEQ-4, CHO-2, and CHO-3 were used as specific hybridization probes discriminating *MAGE-1*, *-2*, and *-3*. The codons of the open reading frame are indicated by points.

cultured cell populations of a subset of cells that are haploid for the relevant chromosome.

To evaluate the expression of *MAGE-1* by various normal and tumor cells, we hybridized Northern blots with a probe covering most of the third exon. In contrast with the result observed with tumor cell line MZ2-MEL.3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals (Fig. 5 and Table 1). Ten of 14 melanoma cell lines of other patients were positive to varying degrees. Two of four samples of melanoma tumor tissue, including a metastasis of patient MZ2, were positive, excluding that the expression of the gene represented a tissue culture artifact. Some tumors of other histological types were also positive (Fig. 5 and Table 1). Thus, the *MAGE* gene family is expressed by many melanomas and also by other tumors. However, because the probe cross-hybridizes, there was no clear indication as to which of the three genes were expressed by these cells. We therefore resorted to PCR amplification and hybridization with specific oligonucleotide probes. cDNAs were obtained and amplified by PCR with oligonucleotide primers that corresponded to sequences of exon 3 that were identical for the three *MAGE* genes. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Fig. 4). Control experiments that were done by diluting RNA of melanoma MZ2-MEL.3.0 into RNA from negative cells indicated that under our conditions the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells that were tested by PCR were confirmed to be negative for the expression of the three *MAGE* genes, suggesting a level of expression of $<1/300$ that of the MZ2 melanoma cell line (Fig. 6). Some melanomas expressed *MAGE* genes *1*, *2*, and *3* whereas others expressed only *MAGE-2* and *-3* (Fig. 6 and Table 1). Some of the other tumors also expressed all three genes, whereas others expressed only *MAGE-2* and *-3* or only *MAGE-3*. The *MAGE* gene family, thus, is expressed by a large array of different tumors and not by most normal cells. The *MAGE* genes may participate in tumor transformation or in aspects of tumor progression such as the ability to metastasize. The observation that *MAGE-1* is expressed in several melanomas shows that tumors of different patients can express the same tumor rejection antigen recognized by autologous CTLs.

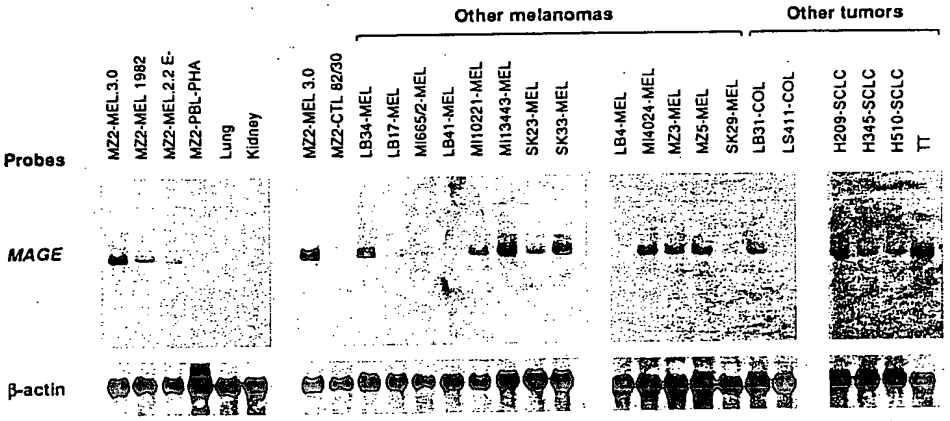

Fig. 5. Northern blot analysis of the expression of *MAGE-1* in tumor cell lines, tumor samples, and normal human tissues. All lanes contained 20 μg of total RNA and were hybridized with a 1.3-kb DNA probe extending from positions 255 to 1544 of exon 3 of *MAGE-1* and with a β-actin probe (*21*). Hybridization with both probes was performed successively on the same blot. Hybridization and washing conditions were the same for all the experiments.

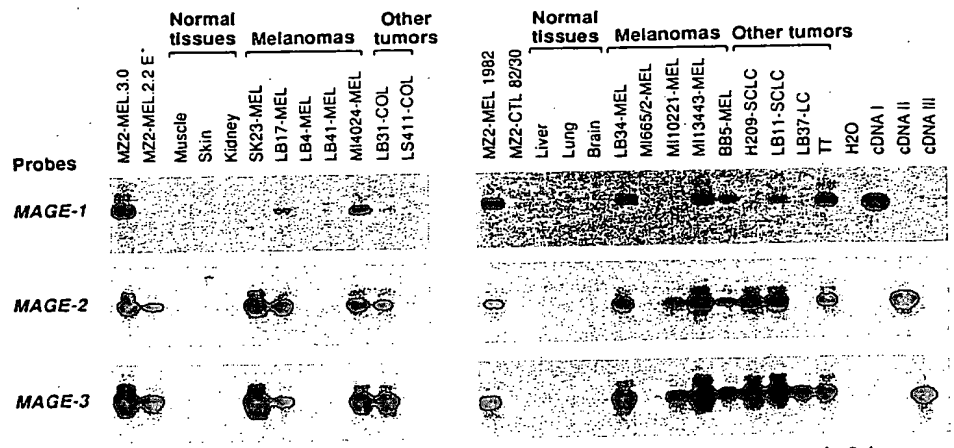

Fig. 6. Detection of the expression of *MAGE-1* and of related genes *MAGE-2* and -3 by reverse transcription and PCR amplification. Total RNA was extracted from the tumor cell lines, tumor samples, and normal human tissues. Oligonucleotides CHO-8 and CHO-9 (Fig. 4), which correspond to identical regions in *MAGE-1*, *-2*, and *-3* were used to prime cDNA synthesis and PCR amplification (*22*). The PCR products were fractionated by size in agarose gels and blotted on nitrocellulose. The blots were hybridized with oligonucleotides probes SEQ-4, CHO-2, and CHO-3, which are highly specific for sequences of *MAGE-1*, *-2*, and *-3*, respectively (Fig. 4). This specificity is demonstrated in the right part of the figure showing hybridization with these three probes of PCR-amplified *MAGE-1*, *-2*, and *-3* cDNA clones. The nitrocellulose filters corresponding to both panels were hybridized, washed, and autoradiographed together.

It is difficult to definitively establish that antigens recognized in vitro on human tu-

Cancer Immunol Immunother (2001) 50:3–15

REVIEW

Nicolina Renkvist · Chiara Castelli
Paul F. Robbins · Giorgio Parmiani

A listing of human tumor antigens recognized by T cells

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Key words Antigens · Tumor · T cells · Epitopes

Complete list of abbreviations of tumor antigens *707-AP* 707 alanine proline · AFP *alpha (α)-fetoprotein* · *ART-4* adenocarcinoma antigen recognized by T cells 4 · *BAGE* B antigen · *β-catenin/m* β-catenin/mutated · *Bcr-abl* breakpoint cluster region–Abelson · *CAMEL* CTL-recognized antigen on melanoma · *CAP-1* carcinoembryonic antigen peptide-1 · *CASP-8* caspase-8 · *CDC27m* cell-division-cycle 27 mutated · *CDK4/m* cycline-dependent kinase 4 mutated · *CEA* carcinoembryonic antigen · *CT* cancer/testis (antigen) · *Cyp-B* cyclophilin B · *DAM* differentiation antigen melanoma (the epitopes of DAM-6 and DAM-10 are equivalent, but the gene sequences are different; DAM-6 is also called MAGE-B2, and DAM-10 is also called MAGE-B1) · *ELF2M* elongation factor 2 mutated · *ETV6-AML1* Ets variant gene 6/acute myeloid leukemia 1 gene · ETS · *G250* glycoprotein 250 · *GAGE* G antigen · *GnT-V* N-acetylglucosaminyltransferase V · *Gp100* glycoprotein 100 kDa · *HAGE* helicose antigen · *HER-2/neu* human epidermal receptor-2/neurological · *HLA-A*0201-R170I* arginine (R) to isoleucine (I) exchange at residue 170 of the α-helix of the α2-domain in the HLA-A2 gene · *HPV-E7* human papilloma virus E7 · *HSP70-2M* heat shock protein 70-2 mutated · *HST-2* human signet ring tumor-2 · *hTERT* or *hTRT* human telomerase reverse transcriptase · *iCE* intestinal carboxyl esterase · *KIAA0205* name of the gene as it appears in databases · *LAGE* L antigen · *LDLR/FUT* low-density lipid receptor/GDP-L-fucose: β-D-galactosidase 2-α-L-fucosyltransferase · *MAGE* melanoma antigen · *MART-1/Melan-A* melanoma antigen recognized by T cells-1/melanoma antigen A · *MC1R* melanocortin 1 receptor · *Myosin/m* myosin mutated · *MUC1* mucin 1 · *MUM-1, -2, -3* melanoma ubiquitous mutated 1, 2, 3 · *NA88-A* NA cDNA clone of patient M88 · *NY-ESO-1* New York-esophagus 1 · *P15* protein 15 · *p190* minor bcr-abl protein of 190 kDa bcr-abl · *Pml/RARα* promyelocytic leukaemia/retinoic acid receptor α · *PRAME* preferentially expressed antigen of melanoma · *PSA* prostate-specific antigen · *PSM* prostate-specific membrane antigen · *RAGE* renal antigen · *RU1* or *RU2* renal ubiquitous 1 or 2 · *SAGE* sarcoma antigen · *SART-1* or *SART-3* squamous antigen rejecting tumor 1 or 3 · *TEL/AML1* translocation Ets-family leukemia/acute myeloid leukemia 1 · *TPI/m* triosephosphate isomerase mutated · *TRP-1* tyrosinase related protein 1, or gp75 · *TRP-2* tyrosinase related protein 2 · *TRP-2/INT2* TRP-2/intron 2 · *WT1* Wilms' tumor gene

Abbreviations used *ALL* acute lymphoblastic leukemia · *AML* acute myeloid leukemia · *APL* acute promyelocytic leukemia · *CML* chronic myelogenous leukemia · *CTL* cytotoxic T lymphocytes · *Ets* E-26 transforming specific (family of transcription factors) · *H/N* head and neck · *MHC* major histocompatibility complex · *NSCLC* non-small cell lung carcinoma · *ORF* open reading frame · *RCC* renal cell carcinoma · *SCC* squamous cell carcinoma · *TSTA* tumor-specific transplantation antigens

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Introduction

Since the cloning of *MAGE-1* [125], the first gene reported to encode a human tumor antigen recognized by T cells, molecular identification and characterization of tumor antigens has mainly been achieved for melanoma. A major reason for this lies in the difficulty of establishing cell lines in vitro from other types of cancer, such lines being necessary to generate tumor-specific CTL

lines or clones to be used in the genetic or biochemical approach aimed at molecularly identifying new cancer antigens. More recently, however, new approaches have allowed the discovery of new antigens recognized by T cells even in tumors other than melanoma.

It is, then, important to categorize these antigens, particularly for the HLA allele restricting their recognition by T cells and for their tissue distribution. With this purpose, tumor antigens have been collected in the present work and briefly commented.

The list presented in the tables below includes all T-cell-defined epitopes encoded by tumor antigens and published by 31 July 2000. Analogs or artificially modified epitopes are excluded from the list. Only tumor antigens recognized by T cells (either cytotoxic CD8+ or helper CD4+) are listed, given their potential importance in the control of tumor growth. Other antigens, identified by antibodies, are excluded but a large collection of them, as detected by the Serex technology, can be found in the data base of the Institute for Cancer Research (www.licr.org/SEREX.htm). It is of note that many tumor antigens (e.g. MAGE, NY-ESO-1a) are now known to be recognized by both T cells and antibodies in the same cancer patients [54].

In the tables herein, tumor antigens are listed in alphabetic order along with the epitope sequence and the HLA allele which restricts recognition by T cells. Furthermore, data on the tissue distribution of each antigen are provided, making this listing an important source for easily retrieving data concerning human tumor antigens.

The listing is meant to be a tool for scientists and students who have an interest in the field of tumor immunology and immunotherapy. The bibliography allows a rapid search for more detailed information at the single antigen or epitope level.

We do not ignore, however, the fact that by recent technologies (e.g., subtractive hybridization, representational-difference analysis, microarrays) hundreds of genes are being detected which are preferentially expressed or overexpressed in neoplastic cells as compared with normal counterparts or are expressed in metastatic but not in primary, early lesions (e.g., melanoma, breast cancer, lymphoma). By using appropriate computer algorithms [9], a number of new epitopes will be identified that can bind MHC molecules. By applying such approaches, a large array of gene products can be screened for their potential antigenic function. More cumbersome may be the selection of the most immunogenic epitopes through appropriate functional assays.

Classification of tumor antigens

Group 1: Class I HLA-restricted cancer/testis antigens (Table 1)

A milestone in tumor immunology was certainly the cloning of *MAGE-1* [125] and the subsequent characterization of the first T-cell-defined antigenic epitope a year later [119]. Those findings were rapidly followed by the identification of new members within this group [6, 123]. The *MAGE*, *BAGE* and *GAGE* families of genes were born. The antigens belonging to this group, now including also NY-ESO-1, were called cancer/testis (CT) antigens for their expression in histologically different human tumors and, among normal tissues, in spermatocytes/spermatogonia of testis and, occasionally, in placenta. These antigens now represent one of the main components for antitumor vaccine development. CT antigens result from reactivation of genes normally silent in adult tissues [27], but that are transcriptionally activated in some tumors [30]. Their expression in testis does not provide targets for an immune reaction because cells of testis do not express class I HLA [56]. Despite the fact that the CT genes are probably the most characterized ones, their physiological function remains largely unknown.

Considering that new genes in the group of CT antigens have been cloned (CT9 [105], CT10 [46], LAGE [72], MAGE-B5, –B6, –C2, –C3 and –D [74, 75], HAGE, SAGE [80]), but that no T-cell epitopes have been identified from them yet, the question arises as to how many more genes encoding CT antigens remain to be discovered and how many epitopes exist that could be of use in cancer immunotherapy.

Group 2: Class I HLA-restricted differentiation antigens (Table 2)

These antigens are shared between tumors and the normal tissue from which the tumor arose; most are found in melanomas and normal melanocytes [2]. Many of these melanocyte lineage-related proteins are involved in the biosynthesis of melanin. Epitopes recognized by both CD8+ and CD4+ T cells can be derived from melanosome proteins [8, 118, 135, 136].

Group 3: Class I HLA-restricted widely expressed antigens (Table 3)

Genes encoding widely expressed tumor antigens have been detected in many normal tissues as well as in histologically different types of tumors with no preferential expression on a certain type of cancer. It is possible that the many epitopes expressed on normal tissues are below the threshold level for T-cell recognition, while their overexpression in tumor cells can trigger an anticancer response even by breaking a previously established tolerance. These widely expressed gene products have revealed a broad spectrum of mechanisms that are involved in generating T-cell-defined epitopes through alterations in gene transcription and translation. To highlight some examples, the epitope of CEA is derived from a non-AUG-defined alternative ORF [1], while the RU2 gene creates its epitope by reverse strand transcription [124].

Table 1 Class I HLA-restricted cancer/testis antigens. All these antigens were found to be expressed by normal spermatocytes and/or spermatogonia of testis. Occasionally *MAGE-3*, *MAGE-4* and the *GAGE* genes were found to be expressed also in placenta [26, 24]. The NY-ESO-1 antigen was found to be expressed in normal ovary cells [18]

Gene	HLA allele	Peptide epitope	Authors [ref.]	Tissue distribution among tumors[a]
MAGE-A1	A1	EADPTGHSY	Traversari et al. 1992 [119]	Melanoma, breast carcinoma, SCLC [27, 29, 125] – sarcoma, NSCLC [27, 29] – thyroid medullary carcinoma [125] – colon carcinoma [27] – laryngeal tumors [29]
MAGE-A1	A3	SLFRAVITK	Chaux et al. 1999a [16]	
MAGE-A1	A24	NYKHCFPEI	Fujie et al. 1999 [37]	
MAGE-A1	A28	EVYDGREHSA	Chaux et al. 1999a [16]	
MAGE-A1, -A2, -A3, -A6	B37	REPVTKAEML	Tanzarella et al. 1999 [113]	Melanoma, colon and breast carcinomas, SCLC [27, 29, 125] – sarcoma, NSCLC [27, 29] – thyroid medullary carcinoma, H/N tumors, bronchial SCC [125] – laryngeal tumors [29] – leukemias [27]
MAGE-A1	B53	DPARYEFLW	Chaux et al. 1999a [16]	Melanoma, breast carcinoma, SCLC [27, 29, 124] – sarcoma, colon carcinoma, NSCLC [27, 29] – thyroid medullary carcinoma [125]
MAGE-A1	Cw2	SAFPTTINF	Chaux et al. 1999a [16]	
MAGE-A1	Cw3	SAYGEPRKL	Chaux et al. 1999a [16]	
MAGE-A1	Cw16	SAYGEPRKL	van der Bruggen et al. 1994b [127]	
MAGE-A2	A2	KMVELVHFL	Visseren et al. 1997 [128]	Melanoma, colon and breast carcinomas, SCLC [27, 29, 125] – sarcoma, NSCLC [27, 29] – thyroid medullary carcinoma [125] – laryngeal tumors [77] – leukemias [27]
MAGE-A2	A2	YLQLVFGIEV	Visseren et al. 1997 [128]	
MAGE-A2	A24	EYLQLVFGI	Tahara et al. 1999 [110]	
MAGE-A3	A1	EADPIGHLY	Gaugler et al. 1994 [40]	Melanoma, colon and breast carcinomas [27, 125] – H/N tumors [18] – bronchial SCC, thyroid medullary and bladder carcinoma, sarcomas, SCLC, NSCLC [125] – leukemias [29]
MAGE-A3	A2	FLWGPRALV	van der Bruggen et al. 1994a [126]	
MAGE-A3	A24	TFPDLESEF	Oiso et al. 1999 [89]	
MAGE-A3	A24	IMPKAGLLI	Tanaka et al. 1997 [111]	
MAGE-A3	B44	MEVDPIGHLY	Herman et al. 1996 [48], Fleischhauer et al. 1996 [35]	
MAGE-A3	B52	WQYFFPVIF	Russo et al. 2000 [103]	
MAGE-A4	A2	GVYDGREHTV	Duffour et al. 1999 [33]	Melanoma, NSCLC, sarcomas, esophageal, colon and breast carcinomas [27]
MAGE-A6	A34	MVKISGGPR	Zorn and Hercend, 1999b [147]	Melanoma, NSCLC, colon carcinoma, leukemias [27]
MAGE-A10	A2	GLYDGMEHL	Huang et al. 1999 [52]	Not defined
MAGE-A12	Cw7	VRIGHLYIL	Panelli et al. 2000 [91], Heidecker et al. 2000 [47]	Melanoma, myeloma, brain tumors, sarcoma, leukemias, SCLC, NSCLC, H/N tumors, bladder, lung, esophageal, breast, prostate and colorectal carcinoma [27]
BAGE	Cw16	AARAVFLAL	Boël et al. 1995 [6]	Melanoma, bladder and mammary carcinomas, H/N SCC, NSCLC, sarcoma
DAM-6, -10	A2	FLWGPRAYA	Fleischhauer et al. 1998 [36]	Melanoma, skin tumors, mammary and ovarian carcinomas [77] – lung carcinoma [25, 77] – seminomas [25]
GAGE-1, -2, -8	Cw6	YRPRPRRY	Van den Eynde et al. 1995 [123], De Backer et al. 1999 [26]	Melanoma, sarcoma, NSCLC, SCLC, mesothelioma, sarcoma, seminoma, leukemias, lymphomas, H/N tumors, bladder, esophageal, mammary, colon, prostate carcinomas
GAGE-3, -4, -5, -6, -7B	A29	YYWPRPRRY	De Backer et al. 1999 [26]	Melanomas, H/N tumors, leukemias, esophageal, lung and bladder carcinomas
NA88-A	B13	MTQGQHFLQKV	Moreau-Aubry et al. 2000 [82]	Melanoma
NY-ESO-1	A2	SLLMWITQCFL	Jäger et al. 1998 [54]	Melanoma, sarcoma, B-lymphomas, hepatoma, H/N tumors, bladder, lung, prostate, ovarian, thyroid and breast carcinoma [18]
NY-ESO-1a (CAG-3)	A2	SLLMWITQC	Jäger et al. 1998 [54]	
	A2	QLSLLMWIT	Jäger et al. 1998 [54]	
	A31	ASGPGGGAPR	Wang et al. 1998b [134]	

[a] Tissue distribution among tumors as described in the given references when different from the paper first reporting the sequence of the epitope

Group 4: Class I HLA-restricted, tumor-specific antigens (Table 4)

Unique tumor antigens arise from point mutations of normal genes (like β-catenin, CDK4) [98, 137], whose molecular changes often accompany neoplastic transformation or progression. These antigens are thus expressed only in the individual tumor where they were identified, since it is unlikely that the same mutation may occur in two different neoplasms unless it involves genes (e.g. RAS) whose alteration is an obligatory step in neoplastic transformation.

Table 2 Class I HLA-restricted melanocyte differentiation antigens. These antigens can only be expressed in normal and neoplastic cells of the same lineage (namely melanocytes, skin, retina, peripheral ganglia) or in normal cells of the prostate gland

Gene	HLA allele	Peptide epitope	Authors [ref.]
MART-1/Melan-A[a]	A2	AAGIGILTV	Coulie et al. 1994 [22], Kawakami et al. 1994a [58]
	A2	EAAGIGILTV	Schneider et al. 1998 [106]
	A2	ILTVILGVL	Castelli et al. 1995 [14]
	B45	AEEAAGIGIL	Schneider et al. 1998 [106]
	B45	AEEAAGIGILT	Schneider et al. 1998 [106]
MC1R	A2	TILLGIFFL	Salazar-Onfray et al. 1997 [104]
	A2	FLALIICNA	Salazar-Onfray et al. 1997 [104]
Gp100	A2	KTWGQYWQV	Bakker et al. 1995 [3]
	A2	AMLGTHTMEV	Tsai et al. 1997 [120]
	A2	MLGTHTMEV	Tsai et al. 1997 [120]
	A2	SLADTNSLAV	Tsai et al. 1997 [120]
	A2	ITDQVPFSV	Kawakami et al. 1995 [61]
	A2	LLDGTATLRL	Kawakami et al. 1994b [59]
	A2	YLEPGPVTA	Cox et al. 1994 [24]
	A2	VLYRYGSFSV	Kawakami et al. 1995 [61]
	A2	RLMKQDFSV	Kawakami et al. 1998 [62]
	A2	RLPRIFCSC	Kawakami et al. 1998 [62]
	A3	LIYRRRLMK	Kawakami et al. 1998 [62]
	A3	ALNFPGSQK	Kawashima et al. 1998 [65]
	A3	SLIYRRRLMK	Kawashima et al. 1998 [65]
	A3	ALLAVGATK	Skipper et al. 1996 [108]
	A24	VYFFLPDHL	Robbins et al. 1997 [99]
	Cw8	SNDGPTLI	Castelli et al. 1999 [15]
PSA	A1	VSHSFPHPLY	Corman et al. 1998 [20]
	A2	FLTPKKLQCV	Correale et al. 1997 [21]
	A2	VISNDVCAQV	Correale et al. 1997 [21]
PSM	A1	HSTNGVTRIY	Corman et al. 1998 [20]
Tyrosinase	A1	KCDICTDEY	Kittlesen et al. 1998 [68]
	A1	SSDYVIPIGTY	Kawakami et al. 1998 [62]
	A2	YMDGTMSQV	Wölfel et al. 1994 [137]
	A2	MLLAVLYCL	Wölfel et al. 1994 [137]
	A24	AFLPWHRLF	Kang et al. 1995 [57]
	B44	SEIWRDIDF	Brichard et al. 1996 [10]
TRP-1 (or gp75)	A31	MSLQRQFLR	Wang et al. 1996b [132]
TRP-2	A2	SVYDFFVWL	Parkhurst et al. 1998 [92]
	A2	TLDSQVMSL	Noppen et al. 2000 [86]
	A31	LLGPGRPYR	Wang et al. 1996a [131]
	A33	LLGPGRPYR	Wang et al. 1998a [133]
	Cw8	ANDPIFVVL	Castelli et al. 1999 [15]

[a] Two different groups simultaneously discovered this gene and gave it two different names, MART-1 and Melan-A respectively

Table 3 Class I HLA-restricted widely expressed antigens

Gene	HLA	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
ART-4	A24	AFLRHAAL DYPSLSATDI	SCC, SCLC, H/N tumors, leukemia, lung, esophageal, gastric, cervical, endometrial, ovarian and breast carcinomas	Testis, placenta, fetal liver	Kawano et al. 2000 [64]
CAMEL	A2	MLMAQEALAFL	Melanoma	Testis, placenta, heart, skeletal muscle, pancreas	Aarnoudse et al. 1999 [1]
CEA	A2	YLSGANLNL (CAP-1)[a]	Melanoma	Testis, placenta, heart, skeletal muscle, pancreas	Tsang et al. 1995 [121]
CEA	A3	HLFGYSWYK	Colon, rectum, pancreas, gastric, breast and lung carcinomas	Gastrointestinal embryonic tissue	Kawashima et al. 1999 [66]
Cyp-B	A24	KFHRVIKDF DFMIQGGDF	Lung adenocarcinoma, T cell leukemia, lymphosarcoma – bladder, ovarian, uterine and esophageal SCC	Ubiquitously expressed in normal tissues	Gomi et al. 1999 [42]

Table 3 (Continued)

Gene	HLA	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
HER2/neu	A2	KIFGSLAFL	Melanoma – ovarian and breast carcinomas	Epithelial cells	Fisk et al. 1995 [34]
HER2/neu	A2	IISAVVGIL	Melanoma, ovarian, pancreatic [96][b] and breast carcinomas	Epithelial cells	Peoples et al. 1995 [95]
HER2/neu	A2	RLLQETELV	Melanoma, ovarian, gastric, pancreatic [96] and breast carcinomas	Epithelial cells	Kono et al. 1998 [71]
HER2/neu	A2	VVLGVVFGI ILHNGAYSL YMIMVKCWMI	Melanoma, ovarian, gastric, pancreatic [96] and breast carcinomas	Epithelial cells	Rongcun et al. 1999 [101]
HER2/neu	A3	VLRENTSPK	Melanoma, ovarian, gastric, pancreatic [96] and breast carcinomas	Epithelial cells	Kawashima et al. 1999 [66]
hTERT[c]	A2	ILAKFLHWL	Lung and ovarian carcinomas – multiple myeloma, melanoma, sarcoma, acute leukemias, non-Hodgkin's lymphomas	Hematopoietic stem cells and progenitors; germinal center cells; basal keratinocytes; gonadal cells; certain proliferating epithelial cells	Vonderheide et al. 1999 [131]
hTRT[c]	A2	ILAKFLHWL RLVDDFLLV	Lung, prostate and ovarian carcinomas, multiple myeloma, melanoma, sarcoma, acute leukemias, non-Hodgkin's lymphomas	Circulating B cells; germinal center B cells; thymocytes; CD34+ progenitor hemopoietic cells	Minev et al. 2000 [81]
iCE	B7	SPRWWPTCL	RCC	Kidney, colon, small intestine, liver, heart, pituitary gland, adrenal gland, prostate, stomach	Ronsin et al. 1999 [102]
MUC1	A11	STAPPAHGV	Breast and ovarian carcinomas, multiple myeloma, B-cell lymphoma	None[d]	Domenech et al. 1995 [31]
MUC1	A2	STAPPVHNV	Breast and ovarian carcinoma, multiple myeloma, B-cell lymphoma	None[d]	Brossart et al. 1999 [11]
MUC2	A2	LLNQLQVNL MLWGWREHV	Ovary, pancreas and breast mucinous tumors, colon carcinoma of non-mucinous type	Colon, small intestine, bronchus, cervix and gall bladder	Böhm et al. 1998 [7]
PRAME	A24	LYVDSLFFL	Melanoma, H/N and lung SCC, NSCLC [122], RCC, adenocarcinoma, sarcoma, leukemias [122]	Testis, endometrium, ovary, adrenals, kidney, brain, skin	Ikeda et al. 1997 [53]
P15	A24	AYGLDFYIL	Melanoma	Testis, spleen, thymus, liver, kidney, adrenal tissue, lung tissue, retinal tissue	Robbins et al. 1995 [97]
RU1	B51	VPYGSFKHV	Melanoma, renal and bladder carcinomas	Testis, kidney, heart, skin, brain, ovary, liver, lung, lymphocytes, thymus, fibroblasts	Morel et al. 2000 [83]
RU2	B7	LPRWPPPQL	Melanoma, sarcomas leukemia – brain, esophageal and H/N tumors – renal, colon, thyroid, mammary, bladder, prostatic and lung carcinomas	Testis, kidney, liver, urinary bladder	Van den Eynde et al. 1999 [124]
SART-1	A24	EYRGFTQDF	Esophageal, H/N and lung SCC – adenocarcinoma, uterine cancer	Testis, fetal liver	Kikuchi et al. 1999 [67]
SART-1	A*2601	KGSGKMKTE	Esophageal, H/N and lung SCC, adenocarcinoma, uterine cancer	Testis, fetal liver	Shichijo et al. 1998 [107]
SART-3	A24	VYDYNCHVDL AYIDFEMKI	H/N, esophageal and lung SCC, adenocarcinoma, leukemia, melanoma	Lymphoid cells, fibroblasts, testis, fetal liver	Yang et al. 1999 [139]
WT1	A2	RMFPNAPYL	Gastric, colon, lung, breast, ovary, uterine, thyroid and hepatocellular carcinomas – leukemia (including AML, ALL and CML)	Kidney, ovary, testis, spleen	Oka et al. 2000 [90]

[a] CAP-1 is an alternative name of this peptide
[b] Tissue distribution among tumors as described in the given references when different from the paper first reporting the sequence of the epitope
[c] Telomerase is expressed in most human tumors: those listed were shown to be susceptible to lysis by cytotoxic T lymphocytes
[d] All epithelial tissues express mucin-like hyperglycosylated molecules

Table 4 Class I HLA-restricted tumor-specific antigens, including both unique (CDK-4, MUM-1, MUM-2, β-catenin, HLA-A2-R170I, ELF2 m, myosin-m, caspase-8, KIAA0205, HSP70-2m) and shared (CAMEL, TRP-2/INT2, GnT-V, G 250) antigens

Gene	HLA allele	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
AFP	A2	GVALQTMKQ	Hepatocellular carcinoma	Fetal liver	Butterfield et al. 1999 [12]
β-Catenin/m	A24	SYLDSGIHF	Melanoma	None	Robbins et al. 1996 [98]
Caspase-8/m	B35	FPSDSWCYF	H/N tumors	None	Mandruzzato et al. 1997 [78]
CDK-4/m	A2	ACDPHSGHFV	Melanoma	None	Wölfel et al. 1995 [138]
ELF2 M	A68	ETVSEQSNV	Lung SCC	None	Hogan et al. 1998 [50]
GnT-V	A2	VLPDVFIRC(V)[a]	Melanoma, brain tumors, sarcoma	Breast and brain (low expression)	Guilloux et al. 1996 [45]
G250	A2	HLSTAFARV	RCC, colon, ovarian and cervical carcinomas	None	Vissers et al. 1999 [129]
HSP70-2M	A2	SLFEGIDIY	RCC, melanoma, neuroblastoma	None	Gaudin et al. 1999 [39]
*HA-A*0201-R170I*	A2	CVEWLRIYLENGK	RCC	None	Brändle et al. 1996 [9]
HST-2	A31	YSWMDISCWI	Gastric signet cell carcinoma	None	Suzuki et al. 1999 [109]
KIAA0205	B44*03	AEPINIQTV	Bladder cancer	None	Gueguen et al. 1998 [44]
MUM-1	B44	EEKLIVVLF	Melanoma	None	Coulie et al. 1995 [23]
MUM-2	B44	SELFRSGLDY	Melanoma	None	Chiari et al. 1999 [19]
MUM-2	Cw6	FRSGLDSYV	Melanoma	None	Chiari et al. 1999 [19]
MUM-3	A28	EAFIQPITR	Melanoma	None	Baurain et al. 2000 [4]
Myosin/m	A3	KINKNPKYK	Melanoma	None	Zorn and Hercend, 1999a [146]
RAGE	B7	SPSSNRIRNT	Melanoma, sarcomas, mesotheliomas, H/N tumors, bladder, renal, colon and mammary carcinomas	Retina only	Gaugler et al. 1996 [41]
SART-2	A24	DYSARWNEI	H/N and lung SCC, lung adenocarcinoma, RCC, melanoma, brain tumors, esophageal and uterine cancers	None	Nakao et al. 2000 [85]
		AYDFLYNYL			
		SYTRLFLIL			
TRP-2/INT2	A68	EVISCKLIKR	Melanoma	None	Lupetti et al. 1998 [76]
707-AP	A2	RVAALARDA	Melanoma	None[b]	Morioka et al. 1995 [84]

[a] VLPDVFIRC(V) = nonamer and decamer peptides are both recognized by CTLs
[b] This antigen is not expressed in normal cells but, as the tissue of the testis was not tested, it will not become clear to which category the antigen may belong until more information is available

In mouse models unique antigens have been shown to be more immunogenic than the other groups of shared antigens [32]; since unique antigens are responsible for the rejection of tumor transplants in mice, they have been defined as tumor-specific transplantation antigens (TSTA). The unique antigens are the most specific targets for immunotherapy, but this potential advantage must be balanced against the almost total impossibility of their clinical use, as they can induce an immune response only against the original tumor on which they were found.

Other tumor-specific but shared antigens have been described which are generated by alteration in splicing mechanisms and which occur in tumor but not in normal cells, as in the case of TRP-2/INT2 [76].

Group 5: Class II HLA-restricted antigens (Table 5)

Stimulation of the CD4+ T helper cells by tumor antigens is considered to be impaired or absent in cancer patients and this may be the reason of an insufficient immune response to tumors. Therefore the identification of tumor antigen epitopes recognized by such lymphocytes is a crucial step in the long sought improvement of antitumor immune response that may result into clinical efficacy. The first epitope presented by a class II HLA and capable of provoking a CD4+ T-cell response was identified in 1994 in melanoma tyrosinase [117]. Then a gap of 4 years followed during which only one additional epitope was characterized [118], before other genes encoding class II-restricted peptides were discovered. However, as the technical and methodological approaches for identifying CD4+ T-cell epitopes of tumor antigens have become available, an exponential increase in reporting such epitopes has been seen. In fact, since 1998 as many as 27 new class II HLA-restricted epitopes from 14 antigens have been molecularly identified using, among others, Ii-cDNA fusion libraries [135], immunized transgenic mice [145] and biochemical approaches [96].

Table 5 Class II HLA-restricted antigens

Gene	HLA allele	Peptide epitope	Tissue distribution		Reference
			Tumors	Normal tissues	
Epitopes from normal protein antigens					
Annexin II	DRB*0401	DVPKWISIM-TERSVPH	Melanoma	Not done	Li et al. 1998 [73]
Gp100	DRB1*0401	WNRQLYPE-WTEAQRLD	Melanoma	Melanocytes	Li et al. 1998 [73]
MAGE-1, -2, -3, -6	DRB*1301 DRB*1302	LLKYRAREP-VTKAE	Melanoma, lung and breast carcinomas, H/N SCC	Testis, placenta	Chaux et al. 1999a [16]
MAGE-3	DR*1101	TSYVKVLHHM-VKISG	Melanoma, lung and breast carcinomas, H/N SCC	Testis, placenta	Manici et al. 1999 [79]
MAGE-3	DRB*1301 DRB*1302	AELVHFLLLK-YRAR	Melanoma, lung and breast carcinomas, H/N SCC	Testis, placenta	Chaux et al. 1999b [17]
MART-1/Melan-A	DRB1*0401	RNGYRALMDKS-LHVGTQCALTRR	Melanoma	Melanocytes	Zarour et al. 2000 [143]
MUC1	DR3	PGSTAPPAHGVT	Breast and ovarian cancers, multiple myeloma, B-cell lymphoma	None[a]	Hiltbold et al. 1998 [49]
NY-ESO-1	DRB4*0101	VLLKEFTVSG	Melanoma, B-lymphoma, hepatoma [18][b], sarcoma, H/N tumors, – bladder, lung, prostate, ovarian, thyroid and breast carcinomas	Testis	Zeng et al. 2000 [145]
NY-ESO-1	DRB4*0101–0103	PLPVPGVLLK-EFTVSGNI VLLKEFTVSG-NILTIRLT AADHRQLQL-SISSCLQQL	B-lymphoma, melanoma, sarcoma, H/N tumors, hepatoma [18] – bladder, lung, prostate, ovarian, thyroid and breast carcinomas	Testis	Jäger et al. 2000 [55]
PSA	DR4	ILLGRMSLFM-PEDTG SLFHPEDTGQVFQ QVFQVSHSFPHPLYD NDLMLLRLSEPAELT KKLQCVQLHVISM GVLQGITSMGSEPCA	Melanoma	Melanocytes	Corman et al. 1998 [20]
Tyrosinase	DRB1*0401	QNILLSNAPLGPQFP DYSYLQDSDPD-SFQD SYLQDSDPDSFQD	Melanoma	Melanocytes	Topalian et al. 1994 [117], Topalian et al. 1996 [118]
Tyrosinase	DRB1*1501	RHRPLQEVYP-EANAPIGHNRE	Melanoma	Melanocytes	Kobayashi et al. 1998a [69]
Tyrosinase	DRB1*0405	EIWRDIDFAHE	Melanoma	Melanocytes	Kobayashi et al. 1998b [70]
Epitopes from mutated protein antigens					
HPV-E7	DR*0401 DR*0407	LFMDTLSFVCPLC LFMDSLNFVCPWC	Cervical carcinoma	None	Höhn et al. 1999 [51]
CDC27/m	DRB1*0401	FSWAMDLDPKGA	Melanoma	None	Wang et al. 1999a [135]
TPI/m	DRB1*0101	GELIGILNAAKVPAD	Melanoma	None	Pieper et al. 1999 [96]

[a] All epithelial tissues express highly glycosilated mucins whereas tumor cells often show hypoglycosilated mucins with a normal protein sequence

[b] Tissue distribution among tumors as described in the given references when different from the paper first reporting the sequence of the epitope

It is of note that even class II-restricted antigens include a subgroup of mutated proteins which, therefore, represent truly tumor-specific antigens.

Group 6: Fusion proteins (Table 6)

In several malignancies, particularly in some forms of leukemia, the molecular mechanism of carcinogenesis involves translocation of chromosomes which results in fusion of distant genes. This often causes the synthesis of fusion proteins which characterize each type of disease (e.g., bcr-abl and pml-RARα in CML and APL, respectively) and generate new epitopes that can be recognized by T cells, either CD8+ or CD4+ in class I or class II HLA restriction, respectively. Although these epitopes appear to be weakly immunogenic in leukemia patients [28], some of these peptides or proteins

Table 6 Epitopes derived from fusion proteins (fusion proteins are never found in normal tissues)

Gene	HLA allele	Peptide epitope	Tissue distribution among tumors	Reference
HLA class I restricted epitopes				
bcr-abl[a]	A2	FMVELVEGA KLSEQESLL MLTNSCVKL	CML	Buzyn et al. 1997 [13]
bcr-abl p210(b3a2)	A2	SSKALQRPV	CML	Yotnda et al. 1998a [141]
bcr-abl (b3a2)	A3	ATGFKQSSK KQSSKALQR	CML	Greco et al. 1996 [43]
bcr-abl p210 (b3a2)	A3, A11	HSATGFKQSSK	CML	Bocchia et al. 1996 [5]
bcr-abl p210(b3a2)	A3	KQSSKALQR	CML	Norbury et al. 2000 [87]
bcr-abl p210(b3a2)	B8	GFKQSSKAL	CML	Norbury et al. 2000 [87]
ETV6/AML	A2	RIAECILGM	ALL	Yotnda et al. 1998b [142]
HLA class II restricted epitopes				
bcr-abl p190 (e1a2)	DRB1*1501	EGAFHGDAEALQRPVAS	ALL	Tanaka et al. 2000 [112]
bcr-abl p210 (b2a2)	DRB5*0101	IPLTINKEEALQRPVAS	CML	ten Bosch et al. 1999 [116]
bcr-abl p210 (b3a2)	DRB1*0401	ATGFKQSSKALQRPVAS	CML	ten Bosch et al. 1996 [115]
bcr-abl p210 (b3a2)	DRB1*1501	ATGFKQSSKALQRPVAS	CML	ten Bosch et al. 1996 [115]
bcr-abl (b3a2)	DRB1*0901	ATGFKQSSKALQRPVAS	CML	Yasukawa et al. 1998 [140]
bcr-abl (b3a2)	DRB1*1101	LIVVIVHSATGFKQSS- KALQRPVA	CML	Pawelec et al. 1996 [93]
bcr-abl (b3a2)	DR11	IVHSATGFKQSSKALQRP- VASDFEP	CML	Bocchia et al. 1996 [5]
Dek-cain	DRB4*0103	TMKQICKKEIRRLHQY	AML	Ohminami et al. 1999 [88]
LDLR/FUT	DRB1*0101	GGAPPVTWRRAPAPG WRRAPAPGAKAMAPG	Melanoma	Wang et al. 1999b [132]
Pml/RARα	DR11	NSNHVASGA- GEAAIETQSSSSEEIV [28]	APL	Gambacorti-Passerini et al. 1993 [38]
p190 minor bcr-abl (e1a2)	DRB1*1501	EGAFHGDAEALQRPVAS	AML	Tanaka et al. 2000 [112]
TEL/AML1	DP5, DP17	IGRIAECILGMNPSR	AML	Yun et al. 1999 [143]

[a]These bcr-abl epitopes are not true fusion proteins generated-epitopes, because they derive from outside the bcr-abl junction

Table 7 Frequency of epitopes recognized by a given HLA allele

Antigen	No. of epitopes	HLA-A	HLA-B	HLA-C
MAGE-1, -2, -3, -4, -6, -10, -12	24	13 (54%)	7 (29%	4 (17%)
GAGE-1, -2, -3, -4, -5, -6, -7B, -8	8	5 (62.5%)	0	3 (37.5%)
MART-1	6	4 (67%)	2 (33%)	0
Gp100	12	11 (92%)	0	1 (8%)
Tyrosinase	6	5 (83%)	1 (17%)	0

can nevertheless be used to pulse dendritic cells for vaccination.

Frequency of epitopes recognized by a given HLA allele (Table 7)

In Table 7 we have summarized, for those antigens from which a high number of epitopes have been described (e.g., CT and differentiation antigens of melanoma) the distribution of epitopes recognized in the context of different HLA loci. This table shows that the majority of epitopes are seen as restricted by HLA-A in all the three groups of antigens considered. Whether this reflects a bias caused by the fact that most of the studies have been carried out with HLA-A-restricted T cells or is mediated by the immunodominant role of the HLA locus in recognition of tumor antigens remains to be established.

Conclusions

Several excellent and timely reviews on tumor antigens have been published periodically during the past few years [8, 63, 100]. However, to our knowledge a comprehensive list of all available tumor antigens and their epitopes and HLA restriction has never been reported, despite the fact that the features of each antigen can be easily found in data bases. We hope that our work may be of interest for many tumor immunologists and students. Needless to say, we may have inadvertently missed information on some antigens despite our careful scrutiny of the published literature; therefore, we will be grateful to any readers who provide us with any missing information. We now plan to update these tables bimonthly in order to keep our data base as informative as possible. The antigen list can also be found at the INT website (www.istitutotumori.mi.it).

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References

1. Aarnoudse CA, van den Doel PB, Heemskerk B, Schrier PI (1999) Interleukin-2-induced, melanoma-specific T cells recognize CAMEL, an unexpected translation product of LAGE-1. Int J Cancer 82: 442
2. Anichini A, Maccalli C, Mortarini R, Salvi S, Mazzocchi A, Squarcina P, Herlyn M, Parmiani G (1993) Melanoma cells and normal melanocytes share antigens recognized by HLA-A2-restricted cytotoxic T cell clones from melanoma patients. J Exp Med 177: 989
3. Bakker AB, Schreurs MW, Tafazzul G, de Boer AJ, Kawakami Y, Adema GJ, Figdor CG (1995) Identification of a novel peptide derived from the melanocyte-specific gp100 antigen as the dominant epitope recognized by an HLA-A2.1-restricted anti-melanoma CTL line. Int J Cancer 62: 97
4. Baurain JF, Colau D, van Baren N, Landry C, Martelange V, Vikkula M, Boon T, Coulie PG (2000) High frequency of autologous anti-melanoma CTL directed against an antigen generated by a point mutation in a new helicase gene. J Immunol 164: 6057
5. Bocchia M, Korontsvit T, Xu Q, Mackinnon S, Yang SY, Sette A, Scheinberg DA (1996) Specific human cellular immunity to bcr-abl oncogene-derived peptides. Blood 87: 3587
6. Boel P, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, Boon T, van der Bruggen P (1995) BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity 2: 167
7. Bohm CM, Hanski ML, Stefanovic S, Rammensee HG, Stein H, Taylor-Papadimitriou J, Riecken EO, Hanski C (1998) Identification of HLA-A2-restricted epitopes of the tumor-associated antigen MUC2 recognized by human cytotoxic T cells. Int J Cancer 75: 688
8. Boon T, van der Bruggen P (1996) Human tumor antigens recognized by T lymphocytes. J Exp Med 183: 725
9. Brandle D, Brasseur F, Weynants P, Boon T, Van den Eynde B (1996) A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma. J Exp Med 183: 2501
10. Brichard VG, Herman J, Van Pel A, Wildmann C, Gaugler B, Wolfel T, Boon T, Lethe B (1996) A tyrosinase nonapeptide presented by HLA-B44 is recognized on a human melanoma by autologous cytolytic T lymphocytes. Eur J Immunol 26: 224
11. Brossart P, Heinrich KS, Stuhler G, Behnke L, Reichardt VL, Stevanovic S, Muhm A, Rammensee HG, Kanz L, Brugger W (1999) Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. Blood 93: 4309
12. Butterfield LH, Koh A, Meng W, Vollmer CM, Ribas A, Dissette V, Lee E, Glaspy JA, McBride WH, Economou JS (1999) Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from alpha-fetoprotein. Cancer Res 59: 3134
13. Buzyn A, Ostankovitch M, Zerbib A, Kemula M, Connan F, Varet B, Guillet JG, Choppin J (1997) Peptides derived from the whole sequence of BCR-ABL bind to several class I molecules allowing specific induction of human cytotoxic T lymphocytes. Eur J Immunol 27: 2066
14. Castelli C, Storkus WJ, Maeurer MJ, Martin DM, Huang EC, Pramanik BN, Nagabhushan TL, Parmiani G, Lotze MT (1995) Mass spectrometric identification of a naturally processed melanoma peptide recognized by CD8+ cytotoxic T lymphocytes. J Exp Med 181: 363
15. Castelli C, Tarsini P, Mazzocchi A, Rini F, Rivoltini L, Ravagnani F, Gallino F, Belli F, Parmiani G (1999) Novel HLA-Cw8-restricted T cell epitopes derived from tyrosinase-related protein-2 and gp100 melanoma antigens. J Immunol 162: 1739
16. Chaux P, Luiten R, Demotte N, Vantomme V, Stroobant V, Traversari C, Russo V, Schultz E, Cornelis GR, Boon T, van der Bruggen P (1999a) Identification of five MAGE-A1 epitopes recognized by cytolytic T lymphocytes obtained by in vitro stimulation with dendritic cells transduced with MAGE-A1. J Immunol 163: 2928
17. Chaux P, Vantomme V, Stroobant V, Thielemans K, Corthals J, Luiten R, Eggermont AM, Boon T, van der Bruggen P (1999b) Identification of MAGE-3 epitopes presented by HLA-DR molecules to CD4(+) T lymphocytes. J Exp Med 189: 767
18. Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ (1997) A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci USA 94: 1914
19. Chiari R, Foury F, De Plaen E, Baurain JF, Thonnard J, Coulie PG (1999) Two antigens recognized by autologous cytolytic T lymphocytes on a melanoma result from a single point mutation in an essential housekeeping gene. Cancer Res 59: 5785
20. Corman JM, Sercarz EE, Nanda NK (1998) Recognition of prostate-specific antigenic peptide determinants by human CD4 and CD8 T cells. Clin Exp Immunol 114: 166
21. Correale P, Walmsley K, Nieroda C, Zaremba S, Zhu M, Schlom J, Tsang KY (1997) In vitro generation of human cytotoxic T lymphocytes specific for peptides derived from prostate-specific antigen. J Natl Cancer Inst 89: 293
22. Coulie PG, Brichard V, Van Pel A, Wolfel T, Schneider J, Traversari C, Mattei S, De Plaen E, Lurquin C, Szikora JP, Renauld JC, Boon T (1994) A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 180: 35
23. Coulie PG, Lehmann F, Lethe B, Herman J, Lurquin C, Andrawiss M, Boon (1995) A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc Natl Acad Sci USA 92: 7976
24. Cox AL, Skipper J, Chen Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CL Jr (1994) Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science 264: 716
25. Dabovic B, Zanaria E, Bardoni B, Lisa A, Bordignon C, Russo V, Matessi C, Traversari C, Camerino G (1995) A family of rapidly evolving genes from the sex reversal critical region in Xp21. Mamm Genome 6: 571
26. De Backer O, Arden KC, Boretti M, Vantomme V, De Smet C, Czekay S, Viars CS, De Plaen E, Brasseur F, Chomez P, Van den Eynde B, Boon T, van der Bruggen P (1999) Characterization of the GAGE genes that are expressed in various human cancers and in normal testis. Cancer Res 59: 3157
27. De Plaen E, Arden K, Traversari C, Gaforio JJ, Szikora JP, De Smet C, Brasseur F, van der Bruggen P, Lethe B, Lurquin C, Brasseur R, Chomez P, De Backer O, Cavenee W, Boon T (1994) Structure, chromosomal localization, and expression of 12 genes of the MAGE family. Immunogenetics 40: 360
28. Dermime S, Bertazzoli C, Marchesi E, Ravagnani F, Blaser K, Corneo GM, Pogliani E, Parmiani G, Gambacorti-Passerini C (1996) Lack of T-cell-mediated recognition of the fusion region of the pml/RAR-alpha hybrid protein by lymphocytes of acute promyelocytic leukemia patients. Clin Cancer Res 2: 593
29. De Smet C, Lurquin C, van der Bruggen P, De Plaen E, Brasseur F, Boon T (1994) Sequence and expression pattern of the human MAGE2 gene. Immunogenetics 39: 121
30. De Smet C, Lurquin C, Lethe B, Martelange V, Boon T (1999) DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. Mol Cell Biol 19: 7327
31. Domenech N, Henderson RA, Finn OJ (1995) Identification of an HLA-A11-restricted epitope from the tandem repeat

domain of the epithelial tumor antigen mucin. J Immunol 155: 4766
32. Dudley ME, Roopenian DC (1996) Loss of a unique tumor antigen by cytotoxic T lymphocyte immunoselection from a 3-methylcholanthrene-induced mouse sarcoma reveals secondary unique and shared antigens. J Exp Med 184: 441
33. Duffour MT, Chaux P, Lurquin C, Cornelis G, Boon T, van der Bruggen P (1999) A MAGE-A4 peptide presented by HLA-A2 is recognized by cytolytic T lymphocytes. Eur J Immunol 29: 3329
34. Fisk B, Blevins TL, Wharton JT, Ioannides CG (1995) Identification of an immunodominant peptide of HER-2/neu protooncogene recognized by ovarian tumor-specific cytotoxic T lymphocyte lines. J Exp Med 181: 2109
35. Fleischhauer K, Fruci D, Van Endert P, Herman J, Tanzarella S, Wallny HJ, Coulie P, Bordignon C, Traversari C (1996) Characterization of antigenic peptides presented by HLA-B44 molecules on tumor cells expressing the gene MAGE-3. Int J Cancer 68: 622
36. Fleischhauer K, Gattinoni L, Dalerba P, Lauvau G, Zanaria E, Dabovic B, van Endert PM, Bordignon C, Traversari C (1998) The DAM gene family encodes a new group of tumor-specific antigens recognized by human leukocyte antigen A2-restricted cytotoxic T lymphocytes. Cancer Res 58: 2969
37. Fujie T, Tahara K, Tanaka F, Mori M, Takesako K, Akiyoshi T (1999) A MAGE-1-encoded HLA-A24-binding synthetic peptide induces specific anti-tumor cytotoxic T lymphocytes. Int J Cancer 80: 169
38. Gambacorti-Passerini C, Grignani F, Arienti F, Pandolfi PP, Pelicci PG, Parmiani G (1993) Human CD4 lymphocytes specifically recognize a peptide representing the fusion region of the hybrid protein pml/RAR alpha present in acute promyelocytic leukemia cells. Blood 81: 1369
39. Gaudin C, Kremer F, Angevin E, Scott V, Triebel F (1999) A hsp70–2 mutation recognized by CTL on a human renal cell carcinoma. J Immunol 162: 1730
40. Gaugler B, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethe B, Brasseur F, Boon T (1994) Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med 179: 921
41. Gaugler B, Brouwenstijn N, Vantomme V, Szikora JP, Van der Spek CW, Patard JJ, Boon T, Schrier P, Van den Eynde BJ (1996) A new gene coding for an antigen recognized by autologous cytolytic T lymphocytes on a human renal carcinoma. Immunogenetics 44: 323
42. Gomi S, Nakao M, Niiya F, Imamura Y, Kawano K, Nishizaka S, Hayashi A, Sobao Y, Oizumi K, Itoh K (1999) A cyclophilin B gene encodes antigenic epitopes recognized by HLA-A24-restricted and tumor-specific CTLs. J Immunol 163: 4994
43. Greco G, Fruci D, Accapezzato D, Barnaba V, Nisini R, Alimena G, Montefusco E, Vigneti E, Butler R, Tanigaki N, Tosi R (1996) Two brc-abl junction peptides bind HLA-A3 molecules and allow specific induction of human cytotoxic T lymphocytes. Leukemia 10: 693
44. Gueguen M, Patard JJ, Gaugler B, Brasseur F, Renauld JC, Van Cangh PJ, Boon T, Van den Eynde BJ (1998) An antigen recognized by autologous CTLs on a human bladder carcinoma. J Immunol 160: 6188
45. Guilloux Y, Lucas S, Brichard VG, Van Pel A, Viret C, De Plaen E, Brasseur F, Lethe B, Jotereau F, Boon T (1996) A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the N-acetylglucosaminyltransferase V gene. J Exp Med 183: 1173
46. Gure AO, Stockert E, Arden KC, Boyer AD, Viars CS, Scanlan MJ, Old LJ, Chen YT (2000) CT10: a new cancer-testis (CT) antigen homologous to CT7 and the MAGE family, identified by representational-difference analysis. Int J Cancer 85: 726
47. Heidecker L, Brasseur F, Probst-Kepper M, Gueguen M, Boon T, Van den Eynde BJ (2000) Cytolytic T lymphocytes raised against a human bladder carcinoma recognize an antigen encoded by gene MAGE-A12. J Immunol 164: 6041
48. Herman J, van der Bruggen P, Luescher IF, Mandruzzato S, Romero P, Thonnard J, Fleischhauer K, Boon T, Coulie PG (1996) A peptide encoded by the human MAGE3 gene and presented by HLA-B44 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE3. Immunogenetics 43: 377
49. Hiltbold EM, Ciborowski P, Finn OJ (1998) Naturally processed class II epitope from the tumor antigen MUC1 primes human CD4+ T cells. Cancer Res 58: 5066
50. Hogan KT, Eisinger DP, Cupp SB 3rd, Lekstrom KJ, Deacon DD, Shabanowitz J, Hunt DF, Engelhard VH, Slingluff CL Jr, Ross MM (1998) The peptide recognized by HLA-A68.2-restricted, squamous cell carcinoma of the lung-specific cytotoxic T lymphocytes is derived from a mutated elongation factor 2 gene. Cancer Res 58: 5144
51. Hohn H, Pilch H, Gunzel S, Neukirch C, Hilmes C, Kaufmann A, Seliger B, Maeurer MJ (1999) CD4+ tumor-infiltrating lymphocytes in cervical cancer recognize HLA-DR-restricted peptides provided by human papillomavirus-E7. J Immunol 163: 5715
52. Huang LQ, Brasseur F, Serrano A, De Plaen E, van der Bruggen P, Boon T, Van Pel A (1999) Cytolytic T lymphocytes recognize an antigen encoded by MAGE-A10 on a human melanoma. J Immunol 162: 6849
53. Ikeda H, Lethe B, Lehmann F, van Baren N, Baurain JF, de Smet C, Chambost H, Vitale M, Moretta A, Boon T, Coulie PG (1997) Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity 6: 199
54. Jager E, Chen YT, Drijfhout JW, Karbach J, Ringhoffer M, Jager D, Arand M, Wada H, Noguchi Y, Stockert E, Old LJ, Knuth A (1998) Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J Exp Med 187: 265
55. Jager E, Jager D, Karbach J, Chen YT, Ritter G, Nagata Y, Gnjatic S, Stockert E, Arand M, Old LJ, Knuth A (2000) Identification of NY-ESO-1 epitopes presented by human histocompatibility antigen (HLA)-DRB4*0101–0103 and recognized by CD4(+) T lymphocytes of patients with NY-ESO-1-expressing melanoma. J Exp Med 191: 625
56. Jassim A, Ollier W, Payne A, Biro A, Oliver RT, Festenstein H (1989) Analysis of HLA antigens on germ cells in human semen. Eur J Immunol 19: 1215
57. Kang X, Kawakami Y, el-Gamil M, Wang R, Sakaguchi K, Yannelli JR, Appella E, Rosenberg SA, Robbins PF (1995) Identification of a tyrosinase epitope recognized by HLA-A24-restricted, tumor-infiltrating lymphocytes. J Immunol 155: 1343
58. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA (1994a) Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA 91: 3515
59. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, Yannelli JR, Adema GJ, Miki T, Rosenberg SA (1994b) Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci USA 91: 6458
60. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JR, Appella E, Rosenberg SA (1994c) Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. J Exp Med 180: 347
61. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, Robbins PF, Sette A, Appella E, Rosenberg SA (1995) Recognition of multiple epitopes in the human mela-

noma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol 154: 3961
62. Kawakami Y, Robbins PF, Wang X, Tupesis JP, Parkhurst MR, Kang X, Sakaguchi K, Appella E, Rosenberg SA (1998) Identification of new melanoma epitopes on melanosomal proteins recognized by tumor infiltrating T lymphocytes restricted by HLA-A1, -A2, and -A3 alleles. J Immunol 161: 6985
63. Kawakami Y, Rosenberg SA (1997) Human tumor antigens recognized by T-cells. Immunol Res 16: 313
64. Kawano K, Gomi S, Tanaka K, Tsuda N, Kamura T, Itoh K, Yamada A (2000) Identification of a new endoplasmic reticulum-resident protein recognized by HLA-A24-restricted tumor-infiltrating lymphocytes of lung cancer. Cancer Res 60: 3550
65. Kawashima I, Tsai V, Southwood S, Takesako K, Celis E, Sette A (1998) Identification of gp100-derived, melanoma-specific cytotoxic T-lymphocyte epitopes restricted by HLA-A3 supertype molecules by primary in vitro immunization with peptide-pulsed dendritic cells. Int J Cancer 78: 518
66. Kawashima I, Tsai V, Southwood S, Takesako K, Sette A, Celis E (1999) Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. Cancer Res 59: 431
67. Kikuchi M, Nakao M, Inoue Y, Matsunaga K, Shichijo S, Yamana H, Itoh K (1999) Identification of a SART-1-derived peptide capable of inducing HLA-A24-restricted and tumor-specific cytotoxic T lymphocytes. Int J Cancer 81: 459
68. Kittlesen DJ, Thompson LW, Gulden PH, Skipper JC, Colella TA, Shabanowitz J, Hunt DF, Engelhard VH, Slingluff CL Jr, Shabanowitz JA (1998) Human melanoma patients recognize an HLA-A1-restricted CTL epitope from tyrosinase containing two cysteine residues: implications for tumor vaccine development [published erratum appears in J Immunol 1999; 162(5): 3106]. J Immunol 160: 2099
69. Kobayashi H, Kokubo T, Sato K, Kimura S, Asano K, Takahashi H, Iizuka H, Miyokawa N, Katagiri M (1998a) CD4+ T cells from peripheral blood of a melanoma patient recognize peptides derived from nonmutated tyrosinase. Cancer Res 58: 296
70. Kobayashi H, Kokubo T, Takahashi M, Sato K, Miyokawa N, Kimura S, Kinouchi R, Katagiri M (1998b) Tyrosinase epitope recognized by an HLA-DR-restricted T-cell line from a Vogt-Koyanagi-Harada disease patient. Immunogenetics 47: 398
71. Kono K, Rongcun Y, Charo J, Ichihara F, Celis E, Sette A, Appella E, Sekikawa T, Matsumoto Y, Kiessling R (1998) Identification of HER2/neu-derived peptide epitopes recognized by gastric cancer-specific cytotoxic T lymphocytes. Int J Cancer 78: 202
72. Lethe B, Lucas S, Michaux L, De Smet C, Godelaine D, Serrano A, De Plaen E, Boon T (1998) LAGE-1, a new gene with tumor specificity. Int J Cancer 76: 903
73. Li K, Adibzadeh M, Halder T, Kalbacher H, Heinzel S, Muller C, Zeuthen J, Pawelec G (1998) Tumour-specific MHC-class-II-restricted responses after in vitro sensitization to synthetic peptides corresponding to gp100 and Annexin II eluted from melanoma cells. Cancer Immunol Immunother 47: 32
74. Lucas S, Brasseur F, Boon T (1999) A new MAGE gene with ubiquitous expression does not code for known MAGE antigens recognized by T cells. Cancer Res 59: 4100
75. Lucas S, De Plaen E, Boon T (2000) MAGE-B5, MAGE-B6, MAGE-C2, and MAGE-C3: four new members of the MAGE family with tumor-specific expression. Int J Cancer 87: 55
76. Lupetti R, Pisarra P, Verrecchia A, Farina C, Nicolini G, Anichini A, Bordignon C, Sensi M, Parmiani G, Traversari C (1998) Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. J Exp Med 188: 1005
77. Lurquin C, De Smet C, Brasseur F, Muscatelli F, Martelange V, De Plaen E, Brasseur R, Monaco AP, Boon T (1997) Two members of the human MAGEB gene family located in Xp21.3 are expressed in tumors of various histological origins. Genomics 46: 397
78. Mandruzzato S, Brasseur F, Andry G, Boon T, van der Bruggen P (1997) A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. J Exp Med 186: 785
79. Manici S, Sturniolo T, Imro MA, Hammer J, Sinigaglia F, Noppen C, Spagnoli G, Mazzi B, Bellone M, Dellabona P, Protti MP (1999) Melanoma cells present a MAGE-3 epitope to CD4(+) cytotoxic T cells in association with histocompatibility leukocyte antigen DR11. J Exp Med 189: 871
80. Martelange V, De Smet C, De Plaen E, Lurquin C, Boon T (2000) Identification on a human sarcoma of two new genes with tumor-specific expression. Cancer Res 60: 3848
81. Minev B, Hipp J, Firat H, Schmidt JD, Langlade-Demoyen P, Zanetti M (2000) Cytotoxic T cell immunity against telomerase reverse transcriptase in humans. Proc Natl Acad Sci USA 97: 4796
82. Moreau-Aubry A, Le Guiner S, Labarriere N, Gesnel MC, Jotereau F, Breathnach R (2000) A processed pseudogene codes for a new antigen recognized by a CD8(+) T cell clone on melanoma. J Exp Med 191: 1617
83. Morel S, Levy F, Burlet-Schiltz O, Brasseur F, Probst-Kepper M, Peitrequin AL, Monsarrat B, Van Velthoven R, Cerottini JC, Boon T, Gairin JE, Van den Eynde BJ (2000) Processing of some antigens by the standard proteasome but not by the immunoproteasome results in poor presentation by dendritic cells. Immunity 12: 107
84. Morioka N, Kikumoto Y, Hoon DS, Morton DL, Irie RF (1995) Cytotoxic T cell recognition of a human melanoma derived peptide with a carboxyl-terminal alanine-proline sequence. Mol Immunol 32: 573
85. Nakao M, Shichijo S, Imaizumi T, Inoue Y, Matsunaga K, Yamada A, Kikuchi M, Tsuda N, Ohta K, Takamori S, Yamana H, Fujita H, Itoh K (2000) Identification of a gene coding for a new squamous cell carcinoma antigen recognized by the CTL. J Immunol 164: 2565
86. Noppen C, Levy F, Burri L, Zajac P, Remmel E, Schaefer C, Luscher U, Heberer M, Spagnoli GC (2000) Naturally processed and concealed HLA-A2.1-restricted epitopes from tumor-associated antigen tyrosinase-related protein-2. Int J Cancer 87: 241
87. Norbury LC, Clark RE, Christmas SE (2000) b3a2 BCR-ABL fusion peptides as targets for cytotoxic T cells in chronic myeloid leukaemia. Br J Haematol 109: 616
88. Ohminami H, Yasukawa M, Kaneko S, Yakushijin Y, Abe Y, Kasahara Y, Ishida Y, Fujita S (1999) Fas-independent and nonapoptotic cytotoxicity mediated by a human CD4(+) T-cell clone directed against an acute myelogenous leukemia-associated DEK-CAN fusion peptide. Blood 93: 925
89. Oiso M, Eura M, Katsura F, Takiguchi M, Sobao Y, Masuyama K, Nakashima M, Itoh K, Ishikawa T (1999) A newly identified MAGE-3-derived epitope recognized by HLA-A24-restricted cytotoxic T lymphocytes. Int J Cancer 81: 387
90. Oka Y, Elisseeva OA, Tsuboi A, Ogawa H, Tamaki H, Li H, Oji Y, Kim EH, Soma T, Asada M, Ueda K, Maruya E, Saji H, Kishimoto T, Udaka K, Sugiyama H (2000) Human cytotoxic T-lymphocyte responses specific for peptides of the wild-type Wilms' tumor gene (WT1) product. Immunogenetics 51: 99
91. Panelli MC, Bettinotti MP, Lally K, Ohnmacht GA, Li Y, Robbins P, Riker A, Rosenberg SA, Marincola FM (2000) A tumor-infiltrating lymphocyte from a melanoma metastasis with decreased expression of melanoma differentiation antigens recognizes MAGE-12. J Immunol 164: 4382
92. Parkhurst MR, Fitzgerald EB, Southwood S, Sette A, Rosenberg SA, Kawakami Y (1998) Identification of a shared HLA-A*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). Cancer Res 58: 4895
93. Pawelec G, Max H, Halder T, Bruserud O, Merl A, da Silva P, Kalbacher H (1996) BCR/ABL leukemia oncogene fusion

peptides selectively bind to certain HLA-DR alleles and can be recognized by T cells found at low frequency in the repertoire of normal donors. Blood 88: 2118
94. Peiper M, Goedegebuure PS, Linehan DC, Ganguly E, Douville CC, Eberlein TJ (1997) The HER2/neu-derived peptide p654–662 is a tumor-associated antigen in human pancreatic cancer recognized by cytotoxic T lymphocytes. Eur J Immunol 27: 1115
95. Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ (1995) Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide. Proc Natl Acad Sci USA 92: 432
96. Pieper R, Christian RE, Gonzales MI, Nishimura MI, Gupta G, Settlage RE, Shabanowitz J, Rosenberg SA, Hunt DF, Topalian SL (1999) Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells. J Exp Med 189: 757
97. Robbins PF, el-Gamil M, Li YF, Topalian SL, Rivoltini L, Sakaguchi K, Appella E, Kawakami Y, Rosenberg SA (1995) Cloning of a new gene encoding an antigen recognized by melanoma-specific HLA-A24-restricted tumor-infiltrating lymphocytes. J Immunol 154: 5944
98. Robbins PF, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E, Rosenberg SA (1996) A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med 183: 1185
99. Robbins PF, El-Gamil M, Li YF, Fitzgerald EB, Kawakami Y, Rosenberg SA (1997) The intronic region of an incompletely spliced gp100 gene transcript encodes an epitope recognized by melanoma-reactive tumor-infiltrating lymphocytes. J Immunol 159: 303
100. Robbins P (2000) Immune recognition of cancer – tumor antigens. In: Parmiani G, Lotze MT (eds) Tumor immunology: molecularly defined antigens and clinical applications. Harwood, London
101. Rongcun Y, Salazar-Onfray F, Charo J, Malmberg KJ, Evrin K, Maes H, Kono K, Hising C, Petersson M, Larsson O, Lan L, Appella E, Sette A, Celis E, Kiessling R (1999) Identification of new HER2/neu-derived peptide epitopes that can elicit specific CTL against autologous and allogeneic carcinomas and melanomas. J Immunol 163: 1037
102. Ronsin C, Chung-Scott V, Poullion I, Aknouche N, Gaudin C, Triebel F (1999) A non-AUG-defined alternative open reading frame of the intestinal carboxyl esterase mRNA generates an epitope recognized by renal cell carcinoma-reactive tumor-infiltrating lymphocytes in situ. J Immunol 163: 483
103. Russo V, Tanzarella S, Dalerba P, Rigatti D, Rovere P, Villa A, Bordignon C, Traversari C (2000) Dendritic cells acquire the MAGE-3 human tumor antigen from apoptotic cells and induce a class I-restricted T cell response. Proc Natl Acad Sci USA 97: 2185
104. Salazar-Onfray F, Nakazawa T, Chhajlani V, Petersson M, Karre K, Masucci G, Celis E, Sette A, Southwood S, Appella E, Kiessling R (1997) Synthetic peptides derived from the melanocyte-stimulating hormone receptor MC1R can stimulate HLA-A2-restricted cytotoxic T lymphocytes that recognize naturally processed peptides on human melanoma cells. Cancer Res 57: 4348
105. Scanlan MJ, Altorki NK, Gure AO, Williamson B, Jungbluth A, Chen YT, Old LJ (2000) Expression of cancer-testis antigens in lung cancer: definition of bromodomain testis-specific gene (BRDT) as a new CT gene, CT9. Cancer Lett 150: 155
106. Schneider J, Brichard V, Boon T, Meyer zum Buschenfelde KH, Wolfel T (1998) Overlapping peptides of melanocyte differentiation antigen Melan-A/MART-1 recognized by autologous cytolytic T lymphocytes in association with HLA-B45.1 and HLA-A2.1. Int J Cancer 75: 451
107. Shichijo S, Nakao M, Imai Y, Takasu H, Kawamoto M, Niiya F, Yang D, Toh Y, Yamana H, Itoh K (1998) A gene encoding antigenic peptides of human squamous cell carcinoma recognized by cytotoxic T lymphocytes. J Exp Med 187: 277
108. Skipper JC, Kittlesen DJ, Hendrickson RC, Deacon DD, Harthun NL, Wagner SN, Hunt DF, Engelhard VH, Slingluff CL Jr (1996) Shared epitopes for HLA-A3-restricted melanoma-reactive human CTL include a naturally processed epitope from Pmel-17/gp100. J Immunol 157: 5027
109. Suzuki K, Sahara H, Okada Y, Yasoshima T, Hirohashi Y, Nabeta Y, Hirai I, Torigoe T, Takahashi S, Matsuura A, Takahashi N, Sasaki A, Suzuki M, Hamuro J, Ikeda H, Wada Y, Hirata K, Kikuchi K, Sato N (1999) Identification of natural antigenic peptides of a human gastric signet ring cell carcinoma recognized by HLA-A31-restricted cytotoxic T lymphocytes. J Immunol 163: 2783
110. Tahara K, Takesako K, Sette A, Celis E, Kitano S, Akiyoshi T (1999) Identification of a MAGE-2-encoded human leukocyte antigen-A24-binding synthetic peptide that induces specific antitumor cytotoxic T lymphocytes. Clin Cancer Res 5: 2236
111. Tanaka F, Fujie T, Tahara K, Mori M, Takesako K, Sette A, Celis E, Akiyoshi T (1997) Induction of antitumor cytotoxic T lymphocytes with a MAGE-3-encoded synthetic peptide presented by human leukocytes antigen-A24. Cancer Res 57: 4465
112. Tanaka Y, Takahashi T, Nieda M, Masuda S, Kashiwase K, Ogawa S, Chiba S, Juji T, Hirai H (2000) Generation of HLA-DRB1*1501-restricted p190 minor bcr-abl (e1a2)-specific CD4+ T lymphocytes. Br J Haematol 109: 435
113. Tanzarella S, Russo V, Lionello I, Dalerba P, Rigatti D, Bordignon C, Traversari C (1999) Identification of a promiscuous T-cell epitope encoded by multiple members of the MAGE family. Cancer Res 59: 2668
114. ten Bosch GJ, Toornvliet AC, Friede T, Melief CJ, Leeksma OC (1995) Recognition of peptides corresponding to the joining region of p210BCR-ABL protein by human T cells. Leukemia 9: 1344
115. ten Bosch GJ, Joosten AM, Kessler JH, Melief CJ, Leeksma OC (1996) Recognition of BCR-ABL positive leukemic blasts by human CD4+ T cells elicited by primary in vitro immunization with a BCR-ABL breakpoint peptide. Blood 88: 3522
116. ten Bosch GJ, Kessler JH, Joosten AM, Bres-Vloemans AA, Geluk A, Godthelp BC, van Bergen J, Melief CJ, Leeksma OC (1999) A BCR-ABL oncoprotein p210b2a2 fusion region sequence is recognized by HLA-DR2a restricted cytotoxic T lymphocytes and presented by HLA-DR matched cells transfected with an Ii(b2a2) construct. Blood 94: 1038
117. Topalian SL, Rivoltini L, Mancini M, Markus NR, Robbins PF, Kawakami Y, Rosenberg SA (1994) Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc Natl Acad Sci USA 91: 9461
118. Topalian SL, Gonzales MI, Parkhurst M, Li YF, Southwood S, Sette A, Rosenberg SA, Robbins PF (1996) Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J Exp Med 183: 1965
119. Traversari C, van der Bruggen P, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T (1992) A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J Exp Med 176: 1453
120. Tsai V, Southwood S, Sidney J, Sakaguchi K, Kawakami Y, Appella E, Sette A, Celis E (1997) Identification of subdominant CTL epitopes of the GP100 melanoma-associated tumor antigen by primary in vitro immunization with peptide-pulsed dendritic cells. J Immunol 158: 1796
121. Tsang KY, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Schlom J (1995) Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. J Natl Cancer Inst 87: 982
122. van Baren N, Chambost H, Ferrant A, Michaux L, Ikeda H, Millard I, Olive D, Boon T, Coulie PG (1998) PRAME, a gene encoding an antigen recognized on a human melanoma by

cytolytic T cells, is expressed in acute leukaemia cells. Br J Haematol 102: 1376
123. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T (1995) A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med 182: 689
124. Van den Eynde BJ, Gaugler B, Probst-Kepper M, Michaux L, Devuyst O, Lorge F, Weynants P, Boon T (1999) A new antigen recognized by cytolytic T lymphocytes on a human kidney tumor results from reverse strand transcription. J Exp Med 190: 1793
125. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254: 1643
126. van der Bruggen P, Bastin J, Gajewski T, Coulie PG, Boel P, De Smet C, Traversari C, Townsend A, Boon T (1994a) A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. Eur J Immunol 24: 3038
127. van der Bruggen P, Szikora JP, Boel P, Wildmann C, Somville M, Sensi M, Boon T (1994b) Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing HLA-Cw*1601. Eur J Immunol 24: 2134
128. Visseren MJ, van der Burg SH, van der Voort EI, Brandt RM, Schrier PI, van der Bruggen P, Boon T, Melief CJ, Kast WM (1997) Identification of HLA-A*0201-restricted CTL epitopes encoded by the tumor-specific MAGE-2 gene product. Int J Cancer 73: 125
129. Vissers JL, De Vries IJ, Schreurs MW, Engelen LP, Oosterwijk E, Figdor CG, Adema GJ (1999) The renal cell carcinoma-associated antigen G250 encodes a human leukocyte antigen (HLA)-A2.1-restricted epitope recognized by cytotoxic T lymphocytes. Cancer Res 59: 5554
130. Vonderheide RH, Hahn WC, Schultze JL, Nadler LM (1999) The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity 10: 673
131. Wang RF, Appella E, Kawakami Y, Kang X, Rosenberg SA (1996a) Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J Exp Med 184: 2207
132. Wang RF, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA (1996b) Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J Exp Med 183: 1131
133. Wang RF, Johnston SL, Southwood S, Sette A, Rosenberg SA (1998a) Recognition of an antigenic peptide derived from tyrosinase-related protein-2 by CTL in the context of HLA-A31 and -A33. J Immunol 160: 890
134. Wang RF, Johnston SL, Zeng G, Topalian SL, Schwartzentruber DJ, Rosenberg SA (1998b) A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. J Immunol 161: 3598
135. Wang RF, Wang X, Atwood AC, Topalian SL, Rosenberg SA (1999a) Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen. Science 284: 1351
136. Wang RF, Wang X, Rosenberg SA (1999b) Identification of a novel major histocompatibility complex class II-restricted tumor antigen resulting from a chromosomal rearrangement recognized by CD4(+) T cells. J Exp Med 189: 1659
137. Wolfel T, Van Pel A, Brichard V, Schneider J, Seliger B, Meyer zum Buschenfelde KH, Boon T (1994) Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. Eur J Immunol 24: 759
138. Wolfel T, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer zum Buschenfelde KH, Beach D (1995) A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 269: 1281
139. Yang D, Nakao M, Shichijo S, Sasatomi T, Takasu H, Matsumoto H, Mori K, Hayashi A, Yamana H, Shirouzu K, Itoh K (1999) Identification of a gene coding for a protein possessing shared tumor epitopes capable of inducing HLA-A24-restricted cytotoxic T lymphocytes in cancer patients. Cancer Res 59: 4056
140. Yasukawa M, Ohminami H, Kaneko S, Yakushijin Y, Nishimura Y, Inokuchi K, Miyakuni T, Nakao S, Kishi K, Kubonishi I, Dan K, Fujita S (1998) CD4(+) cytotoxic T-cell clones specific for bcr-abl b3a2 fusion peptide augment colony formation by chronic myelogenous leukemia cells in a b3a2-specific and HLA-DR-restricted manner. Blood 92: 3355
141. Yotnda P, Firat H, Garcia-Pons F, Garcia Z, Gourru G, Vernant JP, Lemonnier FA, Leblond V, Langlade-Demoyen P (1998a) Cytotoxic T cell response against the chimeric p210 BCR-ABL protein in patients with chronic myelogenous leukemia. J Clin Invest 101: 2290
142. Yotnda P, Garcia F, Peuchmaur M, Grandchamp B, Duval M, Lemonnier F, Vilmer E, Langlade-Demoyen P (1998b) Cytotoxic T cell response against the chimeric ETV6-AML1 protein in childhood acute lymphoblastic leukemia. J Clin Invest 102: 455
143. Yun C, Senju S, Fujita H, Tsuji Y, Irie A, Matsushita S, Nishimura Y (1999) Augmentation of immune response by altered peptide ligands of the antigenic peptide in a human CD4+ T-cell clone reacting to TEL/AML1 fusion protein. Tissue Antigens 54: 153
144. Zarour HM, Kirkwood JM, Kierstead LS, Herr W, Brusic V, Slingluff CL Jr, Sidney J, Sette A, Storkus WJ (2000) Melan-A/MART-1(51–73) represents an immunogenic HLA-DR4-restricted epitope recognized by melanoma-reactive CD4(+) T cells. Proc Natl Acad Sci USA 97: 400
145. Zeng G, Touloukian CE, Wang X, Restifo NP, Rosenberg SA, Wang RF (2000) Identification of CD4+ T cell epitopes from NY-ESO-1 presented by HLA-DR molecules. J Immunol 165: 1153
146. Zorn E, Hercend T (1999a) A natural cytotoxic T cell response in a spontaneously regressing human melanoma targets a neoantigen resulting from a somatic point mutation. Eur J Immunol 29: 592
147. Zorn E, Hercend T (1999b) A MAGE-6-encoded peptide is recognized by expanded lymphocytes infiltrating a spontaneously regressing human primary melanoma lesion. Eur J Immunol 29: 602

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A New Era for Cancer Immunotherapy Based on the Genes that Encode Cancer Antigens

Review

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In 1929, reviewing the available information concerning cancer immunotherapy, W. H. Woglom wrote, "It would be as difficult to reject the right ear and leave the left ear intact as it is to immunize against cancer" (1929). Minimal progress had been made since the earliest descriptions of attempts to immunize against cancer by Nooth, the surgeon to the Duke of Kent, who in 1777 inoculated himself with cancer tissue, or the physician to Louis XVIII, who in 1808 injected himself with breast cancer tissue. Knowledge of the cellular immune system was sparse, and as late as 1958 the Journal of Immunology did not list the word "lymphocyte" in its index. Most attempts to develop cancer vaccines involved immunization of cancer patients using either their own or allogeneic tumors along with a variety of nonspecific immune adjuvants. Even as the basic tenets of modern cellular immunology were elucidated, studies of cancer immunotherapy languished at the periphery of respectable science due to a lack of information regarding the molecular identification of the components of the putative immune reaction against human cancers.

In the past decade, however, the convergence of information from basic studies of cellular and molecular immunology and the application of recombinant DNA techniques to produce pharmacologic quantities of biologic molecules normally present only in minute amounts have substantially changed views concerning the immune response to human cancer and have provided the first demonstrations that immune reactions against cancer antigens can lead to the regression of invasive tumors in selected patients. The molecular identification of tumor antigens, their immunodominant peptides, and the T cell receptors that recognize them have placed studies of tumor immunology and immunotherapy in the mainstream of immunologic research.

Methods for the Detection of Tumor Antigens

Four major techniques have been used to identify cancer antigens capable of eliciting cellular immune reactions in humans. The majority of human tumor antigens now known have been identified by the transfection of genomic DNA or cDNA libraries into cells expressing the appropriate MHC molecule, followed by the identification of transfectants using cytokine release or lysis by human T cells with specific antitumor reactivity (Boon, 1993; Rosenberg, 1996). Biochemical approaches have had more limited success in the identification of human cancer antigens. Attempts have been made to elute peptides from tumor cells or from MHC molecules purified from tumor cells and to detect fractions capable of stimulating antitumor T cells after pulsing purified fractions onto antigen-presenting cells. Triple quadrapole mass spectrometric techniques have then been used to sequence the minute quantities of peptides obtained (Cox et al., 1994). This approach has been severely limited by the need for custom-made, highly specialized equipment and the requirement that peptides be present in sufficient quantity to enable their identification by these physical techniques.

The ability of antigen-presenting cells to endocytose proteins and present peptides on class II MHC molecules has been utilized to identify antigens recognized by CD4 T cells, although thus far few antigens have been identified using this approach (Topalian et al., 1994).

Each of the three techniques mentioned above is dependent on the prior availability of T cells capable of recognizing tumor antigens, a requirement that often cannot be met. A fourth approach to the identification of tumor antigens has involved attempts to develop, by in vitro sensitization techniques, T cells against candidate tumor antigens (Parkhurst et al., 1998). T cells successfully generated in vitro against candidate antigens have then been tested for their ability to recognize intact tumor cells, and the presence of such reactivity provides strong evidence that these candidate proteins represent tumor antigens. Genes encoding candidate tumor antigens have been transfected or transduced into antigen-presenting cells or synthesized peptides from candidate antigens based on known MHC-binding motifs have been pulsed onto antigen-presenting cells and used for these in vitro sensitizations.

An alternate technique for the identification of tumor antigens that is also not dependent on the prior availability of antitumor T cells has recently been described. This approach, called serological analysis of recombinant cDNA expression libraries (SEREX), uses diluted serum from cancer patients to detect prokaryotically expressed cDNA libraries prepared from tumors (Sahin et al., 1995; Chen et al., 1997). This approach is based on the assumption that antibody production implies that a helper T cell reaction exists against the detected antigen. At least one tumor antigen, NY-ESO-1, subsequently shown to be reactive with $CD8^+$ T cells, has been identified using this approach (Chen et al., 1997), and other candidate antigens are being studied (Tureci et al., 1996).

Characteristics of Tumor Antigens

An unexpected diversity of mechanisms can result in the generation of antigenic epitopes recognized by tumor-specific T cells. These epitopes have come from normal nonmutated genes whose expression is limited to cancer and selected normal tissues, from aberrantly expressed intronic sequences, from alternative open reading frames of normal genes, and from mutations specific to the individual cancer (Table 1).

Malignant melanomas have been a particularly rich

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Table 1. Defined Cancer Antigens

Antigen Category	Gene	MHC Restriction
Melanocyte differentiation	MART-1/MelanA	A2, B45
	gp100	A2, A3, A24
	Tyrosinase	A1, A2, A24, DR4
	TRP-1	A31
	TRP-2	A2, A31, A68
Cancer testis	MAGE-1	A1, Cw16
	MAGE-3	A1, A2, B44
	GAGE-1/2	Cw16
	BAGE	Cw16
	RAGE	B7
	NY-ESO-1	A2, A31
Tumor specific	CDK-4	A2
	β-catenin	A24
	MUM-1	B44
	Caspase-8	B35
	KIAA0205	B44
	HPVE7	A2
Widely expressed	SART-1	A26
	PRAME	A24
	p15	A24

source of tumor antigens that are found not only on melanomas but on other tumor types as well. Two major categories of normal, non-mutated genes that encode shared tumor antigens have been identified: (1) differentiation antigens shared on melanomas and melanocytes, and (2) differentiation antigens shared on a variety of tumors as well as normal testes.

The expression of one family of differentiation antigens is limited to melanomas as well as melanocytes, the cell of origin of this tumor, and pigment-producing cells in the retina. The T cells used to identify these melanoma/melanocyte differentiation antigens have largely been derived from tumor-infiltrating lymphocytes (TIL) obtained from patients with growing cancers or from autologous mixed-lymphocyte tumor cultures (Boon, 1993; Rosenberg, 1996). The presence of precursors capable of reacting with normal nonmutated self-antigens within the tumor has suggested that the inflammatory microenvironment at the site of the tumor has resulted in the breaking of peripheral tolerance to these antigens. Alternatively, the tumor may have acted as a "sink" for the accumulation of precursors normally present, since precursors to at least one of these antigens has also been found in normal individuals.

The most common antitumor reactivity found in melanoma patients is directed against the MART-1/MelanA antigen, which is a 118–amino acid protein of unknown function that contains a 21–amino acid transmembrane region (Coulie et al., 1994; Kawakami et al., 1994a). The immunodominance of this antigen in HLA-A2 individuals is striking (Kawakami et al., 1994c). Of 29 HLA-$A2^+$ TIL exhibiting HLA-A2-restricted recognition of shared tumor antigens, 21 reacted with the MART-1 protein. In all 21 cases, these TIL recognized the identical 9-mer AAGIGILTV peptide. A MART-1 epitope has also been identified that is restricted by HLA-B45 (Schneider et al., 1998). Responses to the MART-1/MelanA epitope can be readily elicited in vitro from peripheral lymphocytes of normal nonmelanoma-containing individuals (Marincola et al., 1996). MART-1-reactive T cells can react with differing peptides derived from a variety of exogenous antigens including viral proteins, and cross-reactivity with these epitopes may result in the high precursor frequency of T cells against the MART-1 epitope (Loftus et al., 1996).

Of the 29 HLA-A2-reactive TIL, 13 reacted with the gp100 melanoma differentiation antigen (Kawakami et al., 1994b). This 661–amino acid protein was identified independently by screening cDNA expression libraries with melanoma reactive lymphocytes (Kawakami et al., 1994b) as well as by elution of peptides from an HLA-$A2^+$ melanoma (Cox et al., 1994). This gene had previously been isolated and was known to encode a protein recognized by the HMB-45 monoclonal antibody but was unknown as a T cell antigen. The gp100 protein encodes an enzyme involved in melanin synthesis, and multiple gp100 epitopes have been identified restricted not only by HLA-A2 but by HLA-A3 and HLA-A24 as well (Kawakami et al., 1995; Skipper et al., 1996b; Robbins et al., 1997; Tsai et al., 1997; Kawakami et al., 1998). Interestingly, the epitope recognized by HLA-A24-restricted T cells results from an aberrantly spliced product of the gp100 gene, resulting in translation of the fourth intron of the gp100 molecule in both melanomas and in normal melanocytes (Robbins et al., 1997).

Tyrosinase, an enzyme critical for the synthesis of melanin, has been shown to be an antigen recognized by HLA-A1-, HLA-A2-, and HLA-A24-restricted T cells (Brichard et al., 1993; Robbins et al., 1994; Wolfel et al., 1994; Kittlesen et al., 1998). One of the naturally occurring peptide epitopes restricted by HLA-A2 contains a posttranslational modification of an asparagine to aspartic acid residue (Skipper et al., 1996a). Tyrosinase is the only melanoma/melanocyte differentiation antigen known to be recognized by $CD4^+$ tumor-reactive T cells (Topalian et al., 1994). A $CD4^+$ tumor-infiltrating lymphocyte cross-reactive with HLA-$DR0401^+$ melanomas was used to test for reactivity against candidate melanoma antigens previously shown to be recognized by $CD8^+$ T cells, and tyrosinase was identified as the reactive antigen. Several class II–presented epitopes from tyrosinase have also been identified.

Two melanocyte proteins that bear approximately 40% amino acid homology to tyrosinase have been identified as T cell antigens (Wang et al., 1995, 1996, 1998a). TRP-1 is the most prevalent protein present in melanocytes and melanomas and was recognized as a tumor antigen reactive with HLA-A31-restricted TIL 586 from a patient with melanoma. The peptide epitope reactive with TIL 586 was encoded by the normal TRP-1 gene but was not translated from the primary open reading frame (Wang et al., 1996). The third open reading frame of this gene translated a 21–amino acid peptide that contained the 9–amino acid peptide epitope recognized by TIL 586. This alternative open reading frame was also translated in normal melanocytes. At least one other example of an alternative open reading frame encoding a tumor antigen was subsequently described (Wang et al., 1998b), and rare other examples exist of the recognition of the products of alternative open reading frames (Malarkannan et al., 1995; Quelle et al., 1995; Elliott et al., 1996; Bullock et al., 1997). Clones from TIL 586 also recognized TRP-2 as an HLA-A31-restricted antigen,

and an epitope from the normal open reading frame of this protein was identified (Wang et al., 1998a). TRP-1 and TRP-2 epitopes can bind to HLA-A3, -A11, -A31, -A3, and -A68 (Sidney et al., 1996; Wang et al., 1998a).

Studies of the TRP-2 protein represent an example of the use of in vitro sensitization techniques to identify new tumor antigenic epitopes. To identify HLA-A2-restricted TRP-2 epitopes, multiple peptides were synthesized from the TRP-2 protein based on HLA-A2-binding motifs. Using in vitro sensitization techniques against those multiple peptides, an HLA-A2-restricted T cell was generated against a single TRP-2 peptide, SVYDFFVWL, that was also capable of recognizing HLA-$A2^+$ melanomas (Parkhurst et al., 1998).

Thus, at least five melanoma/melanocyte differentiation proteins have been shown to mediate the generation of T cells capable of recognizing melanomas restricted by multiple class I alleles.

Another class of shared differentiation antigens is expressed on tumors as well as germ cells of the testes. The first cloned human tumor antigen recognized by T cells was isolated utilizing T cells from a melanoma patient who had been repeatedly immunized with mutagenized cancer cells (Van der Bruggen et al., 1991). The T cells from this individual were sensitized in vitro to the autologous melanoma, and reactive T cell clones that recognized a protein encoded by a gene termed MAGE-1 were identified. This gene belonged to a multigene family containing at least 12 different genes expressed in a small percentage of melanomas as well as cancers arising in the breast, prostate, esophagus, colon, and lung (Brasseur et al., 1992; Traversari et al., 1992; Boon, 1993; Weynants et al., 1994; Van Pel et al., 1995). Except for testes, the gene does not appear to be expressed in normal tissues. The MAGE-3 gene in this family is of particular interest because it is expressed in a high percentage of melanomas and head and neck squamous cell carcinomas (Gaugler et al., 1994; Van Den Eynde and Van der Bruggen, 1997).

By screening cDNA libraries utilizing T cell clones from the same patient used to identify the MAGE gene family, another series of multigene families called BAGE (Boel et al., 1995) and GAGE (Van Den Eynde et al., 1995) have also been identified. These gene products appear to have similar expression distributions to the MAGE gene family. Epitopes from the MAGE, GAGE, and BAGE genes have been identified that are restricted by HLA-A1, -A2, -B44, and -Cw16 (Traversari et al., 1992; Gaugler et al., 1994; Van der Bruggen et al., 1994a, 1994b; Fleischauer et al., 1996; Tanaka et al., 1997). This family of tumor antigens was detected using in vitro sensitized T cells from the autologous patient, although no descriptions of TIL recognizing these antigens have yet been reported. The levels of MAGE-1 expressed by some tumor cells can be below the level of detection required for lymphocyte recognition (Lethe et al., 1997), even though some studies suggest that as few as five peptides on the cell surface are sufficient for T cell recognition (Brower et al., 1994; Sykulev et al., 1996).

Another antigen expressed in cancers and testes is the NY-ESO-1 antigen that was identified by screening a melanoma cDNA expression library using serum from a patient with squamous cell carcinoma of the esophagus (Chen et al., 1997). Although this antigen was originally identified by its ability to react with a naturally occurring antibody, this gene product was subsequently found to be reactive with T cells as well. By screening clones from melanoma TIL 586, the NY-ESO-1 gene was independently identified and found to encode two different epitopes restricted by HLA-A31, with one encoded by an alternative open reading frame of the NY-ESO-1 gene (Wang et al., 1998b). Approximately 10% of patients develop high-titer antibodies against NY-ESO-1 (Stockert et al., 1998) and an HLA-A2-restricted CTL line from a melanoma patient with a high titer of antibodies to NY-ESO-1 was also found to react with the NY-ESO-1 protein. Two HLA-A2-restricted epitopes have been identified (Jager et al., 1998). NY-ESO-1 appears to be expressed only in the testes and in a wide variety of tumors including melanomas and cancers of the ovary, bladder, breast, prostate, and lung (Chen et al., 1997; Stockert et al., 1998).

The melanocyte/differentiation antigens and the cancer/testis antigens represent the majority of tumor antigens thus far identified that are encoded by normal nonmutated genes. A class of poorly understood tumor antigens encoded by genes that are widely expressed in normal tissues but appear to be selectively presented on tumor cells has been described as well. An HLA-A24-restricted TIL line reactive with HLA-A24 melanomas was used to isolate a previously undescribed gene called p15 (Robbins et al., 1995). Although Northern blots revealed that p15 was expressed in normal tissues, only tumor was recognized by this TIL. The mechanisms involved in this unique tumor recognition have not yet been elucidated. The SART-1 gene had similar characteristics and was recognized in lung and esophageal squamous cell carcinomas but was also expressed in normal proliferating cell lines (Shichijo et al., 1998). The PRAME gene product, reactive with T cells restricted by HLA-A24 that expressed an NK inhibitory receptor, was identified only when the tumor lost the appropriate HLA-Cw7 class I molecule that limited recognition of these tumor cells (Lehmann et al., 1995). This gene was expressed in low levels in a variety of normal tissues, with much greater levels of expression in melanomas. It is thus clear that a variety of normal nonmutated genes can result in the generation of T cells reactive with tumors. These genes come from normal differentiation proteins, from translated alternative open reading frames, and from nonmutated intronic sequences. T cells recognizing these gene products are capable of reacting with a variety of tumor types.

Based on murine tumor models, in which in vivo tumor protection appeared to be unique to the individual immunizing tumor, it was expected that human tumor antigens would represent the products of mutated genes. A variety of gene mutations that give rise to tumor antigenic epitopes in the human have now been described. An HLA-B44-restricted T cell was identified that recognized the product of a gene termed MUM-1 (Coulie et al., 1995). The epitope recognized by this T cell spanned an intron/exon boundary and contained a single base mutation. The normal and mutated peptides bound equally to HLA-B44, and thus the mutation appeared to alter a T cell contact residue.

An HLA-A2-restricted CTL clone was found to recognize the mutated product of a cyclin-dependent kinase 4 (CDK4) gene (Wolfel et al., 1995). A single base mutation in this gene resulted in the substitutions of a cysteine for an arginine in an anchor position of this peptide and altered the binding properties of this peptide to HLA-A2. One hundred-fold lower concentrations of the mutant peptide compared to the native peptide were recognized.

A mutation in the α-helical region of the HLA-A2 molecule gave rise to a mutated epitope recognized by T cells reactive with an autologous renal cell carcinoma (Brandle et al., 1996). Similarly, a point mutation in a gene encoding a protein recognized by T cells reactive with an autologous bladder carcinoma resulted in the unique recognition of the autologous tumor cell line (Gueguen et al., 1998). A single point mutation in the normal stop code encoding the caspase-8 gene resulted in the extension of the normal open reading frame of this gene by 88 amino acids, and a T cell epitope was encoded within this region that was recognized by the T cells of the autologous patient with a squamous cell carcinoma (Mandruzzato et al., 1997). A TIL that recognized an HLA-A24 melanoma antigen was found to recognize a mutated β-catenin gene product. A single point mutation that substituted a phenylalanine for a serine residue gave rise to a peptide epitope that was recognized at 1 million times lower concentration than the normal peptide (Robbins et al., 1996). The same mutation in β-catenin was found at about 10% of all melanomas examined (Rubinfeld et al., 1997).

Development of Cancer Immunotherapy Based on the Identification of Tumor Antigens

Leonardo da Vinci (1452–1519) wrote that "the supreme misfortune is when theory outstrips performance," a situation that now characterizes the current state of cancer immunotherapy. The discovery of tumor antigens and the identification of their immunodominant epitopes has shifted emphasis to the utilization of these antigens to mediate the destruction of growing cancers in humans. Attempts are in progress to develop immunotherapies based on the specific stimulation of immune reactions against defined tumor antigens rather than on the nonspecific stimulation of the immune system that has characterized prior approaches. Although studies of the administration of interleukin-2 to cancer patients have demonstrated that broad stimulation of immune reactivity can lead to the complete and partial regression of even bulky invasive tumors in humans, these clinical effects are limited to a small number of patients with limited histologic types of cancers (Rosenberg et al., 1994, 1998a). Cancer immunotherapy based either on immunization against specific tumor antigens or on the adoptive transfer of in vitro generated lymphocytes with high levels of specific reactivity against tumor antigens represent attractive approaches.

Which tumor antigens represent the best targets for the development of effective immunotherapies? Several lines of evidence exist that the melanoma/melanocyte differentiation antigens can be effective targets for the immune destruction of melanoma. TIL reactive with defined melanoma/melanocyte antigens are capable of mediating tumor regression when adoptively transferred to cancer patients (Kawakami et al., 1995; Rosenberg, 1997). An analysis of HLA-A2-restricted TIL transferred to melanoma patients demonstrated a statistically significant correlation of tumor destruction in vivo and recognition of the gp100 tumor antigen (Kawakami et al., 1995). This association was not seen for TIL reactive with the MART-1 antigen. Further evidence for the role of these melanoma/melanocyte antigens in tumor destruction comes from studies of depigmentation (vitiligo) in cancer patients (Rosenberg and White, 1996). No vitiligo was seen in 102 patients with renal cell cancer treated with IL-2 compared to a 16% incidence of vitiligo in 73 similarly treated melanoma patients. There was no evidence of depigmentation seen in melanoma patients who did not respond to this immunotherapy compared with vitiligo in 29% of patients who showed an anticancer response ($p2 = 0.002$). Thus, tumor destruction was associated with the destruction of normal melanocytes, strongly suggesting that the targets in tumor and normal tissue were the melanoma/melanocyte differentiation antigens.

The downregulation of expression of antigens may represent a problem for the development of immunotherapies because of the possible immunoselection of nonexpressing tumor clones. For this reason an ideal target for immune destruction is a protein that is essential for the malignant phenotype, and mutations that give rise to antigens in such proteins have been described. The antigenic mutation in CDK4 appeared to disrupt the binding of the normal CDK4 inhibitor, p16-INK4a, and disruption of this interaction may have contributed to unregulated tumor cell growth (Wolfel et al., 1995; Sherr, 1996). Loss of expression of this mutation would thus presumably reverse the malignant phenotype. Similarly, the mutated caspase-8 gene product may play a role in the apoptic pathway and thus be involved in tumorigenesis (Mandruzzato et al., 1997). The mutated β-catenin gene product in selected HLA-A24 melanomas appeared to stabilize the β-catenin molecule and promote the formation of complexes with the Tcf family of DNA-binding proteins, which may activate target genes in tumorigenesis (Robbins et al., 1996; Korinek et al., 1997; Rubinfeld et al., 1997; Morin et al., 1999).

Although the mutated proteins may be attractive targets for cancer immunotherapy, they will be appropriate only for the individual patients expressing these mutations and thus will not be suitable for the development of widely applicable cancer immunotherapies. Improvements in methods to identify tumor-associated mutations recognized by the immune system, however, leave open the possibility of tailoring individual immunotherapies for individual cancer patients.

The development of cancer immunotherapies based on the molecular characterization of tumor antigens is in its early stages, and only a small number of clinical trials have been reported. Immunization of patients using recombinant avipox, vaccinia virus, or adenovirus encoding cancer antigens have thus far not resulted in consistent in vivo generation of antitumor T cells or clinical responses, probably due in part to the potent generation of neutralizing antibodies directed against the viral envelope proteins (Tsang et al., 1995; Rosenberg et al., 1998b; Marshall et al., 1999). Preliminary

evidence has suggested that immunization with native or modified (heteroclitic) peptides derived from tumor antigens may be a more promising means of immunization. Of 12 patients immunized with the soluble HLA-A1-binding MAGE-3 peptide, 2 patients exhibited a partial tumor regression, although there was no evidence for the generation of CTL in the peripheral blood of treated patients (Marchand et al., 1995). Although no clinical responses were seen in six patients immunized with HLA-A2-restricted peptides from MART-1, tyrosinase, and gp100 antigens, a modest enhancement of in vitro antipeptide responses were seen (Jager et al., 1996a). These same workers, however, provided preliminary evidence that the addition of GM-CSF administration to the vaccine regimen mediated tumor regression in three patients (Jager et al., 1996b) and that the clinical impact of immune reactions induced by peptide immunization may be limited by the expression of antigens by the tumor (Jager et al., 1996c).

Two recently reported clinical trials, however, have provided encouraging information concerning the ability of peptide immunization to mediate tumor regression. Patients (10 of 11) immunized with a peptide from the gp100 molecule gp100:209–217 (210M), modified to increase binding to HLA-A2, showed a substantial induction of circulating precursors capable of reacting not only with the native peptide but with HLA-A2-matched tumors (Rosenberg et al., 1998c). Although no significant clinical responses were seen in these 11 patients, objective clinical responses were seen in 13 of 42 patients who received this modified gp100:209–217 (210M) peptide in conjunction with IL-2 (Rosenberg et al., 1998c). This response rate was three times that expected from the use of IL-2 alone, and prospective randomized trials comparing the treatment of patients with metastatic melanoma using either IL-2 alone or IL-2 plus peptide immunization are being initiated. In another clinical trial, 16 patients with metastatic melanoma were immunized with dendritic cells pulsed either with immunodominant melanoma peptides or with melanoma lysates, and objective tumor regression was reported in five patients (Nestle et al., 1998). Multiple other approaches to the active immunization of patients using the products of these tumor antigen genes are being explored, including immunization with peptides, intact protein, "naked" DNA, and recombinant adenovirus, vaccinia virus, and fowlpox virus encoding these tumor antigens. The ex vivo exposure of antigen-presenting cells to antigenic proteins or peptides and the administration of immune-enhancing cytokines such as IL-2 and IL-12 in conjunction with these immunizations is also being explored in experimental models and in human clinical trials.

In vitro sensitization techniques using repeated in vitro stimulation against immunodominant peptides or utilizing tumor antigens pulsed onto professional antigen-presenting cells have resulted in the in vitro generation of T cells with extremely high antitumor activity, and clinical protocols utilizing the adoptive transfer of these in vitro sensitized cells are in progress as well.

Marshalling the immune response to reject human tumors is a daunting challenge, although not as difficult as the need to "reject the right ear and leave the left ear intact." A new era of cancer immunotherapy has, however, arrived based on the molecular identification of human cancer antigens.

References

Boel, P., Wildmann, C., Sensi, M.L., Brasseur, R., Renauld, J.C., Coulie, P., Boon, T., and Van der Bruggen, P. (1995). BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity *2*, 167–175.

Boon, T. (1993). Tumor antigens recognized by cytolytic T lymphocytes: present perspectives for specific immunotherapy. Int. J. Cancer *54*, 177–180.

Brandle, D., Brasseur, F., and Weynants, P. (1996). A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma. J. Exp. Med. *183*, 2501–2508.

Brasseur, F., Marchand, M., Vanwijck, R., Herin, M., Lethe, B., Chomez, P., and Boon, T. (1992). Human gene MAGE-1, which codes for a tumor-rejection antigen, is expressed by some breast tumors. Letter to the editor. Int. J. Cancer *52*, 839–841.

Brichard, V., Van Pel, A., Wolfel, T., Wolfel, C., De Plaen, E., Lethe, B., Coulie, P., and Boon, T. (1993). The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J. Exp. Med. *178*, 489–495.

Brower, R.C., England, R., and Takeshita, T. (1994). Minimal requirements for peptide mediated activation of CD8+ CTL. Mol. Immunol. *31*, 1285–1293.

Bullock, T.N., Patterson, A.E., Notidis, E., Franlin, L.L., and Eisenlohr, L.C. (1997). Initiation codon scanthrough versus termination codon readthrough demonstrates strong potential for major histocompatibility complex class I-restricted cryptic epitope expression. J. Exp. Med. *186*, 1051–1058.

Chen, Y.T., Scanlan, M.J., and Sahin, U. (1997). A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc. Natl. Acad. Sci. USA *94*, 1914–1918.

Coulie, P., Brichard, V., Van Pel, A., Wolfel, T., Schneider, J., Traversari, C., Mattei, S., DePlaen, E., Lurquin, C., Szikora, J., et al. (1994). A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J. Exp. Med. *180*, 35–42.

Coulie, P.G., Lehmann, F., Lethe, B., Herman, J., Lurquin, C., Andrawiss, M., and Boon, T. (1995). A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc. Natl. Acad. Sci. USA *92*, 7976–7980.

Cox, A.L., Skipper, J., Chen, Y., Henderson, R.A., Darrow, T.L., Shabanowitz, J., Engelhard, V.H., Hunt, D.F., and Slingluff, C.L. (1994). Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science *264*, 716–719.

Elliott, T., Bodmer, H., and Townsend, A. (1996). Recognition of out-of-frame major histocompatibility complex class I-restricted epitopes in vivo. Eur. J. Immunol. *26*, 1175–1179.

Fleischauer, K., Fruci, D., and Van Endert, P. (1996). Characterization of antigenic peptides presented by HLA-B44 molecules on tumor cells expressing the gene MAGE-3. Int. J. Cancer *68*, 622–628.

Gaugler, B., Van Den Eynde, B., Van der Bruggen, P., Romero, P., Gaforio, J.J., De Plaen, E., Lethe, B., Brassuer, F., and Boon, T. (1994). Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J. Exp. Med. *179*, 921–930.

Gueguen, M., Matard, J.J., and Gaugler, B. (1998). An antigen recognized by autologous CTLs on a human bladder carcinoma. J. Immunol. *160*, 6188–6194.

Jager, E., Bernhard, H., Romero, P., Ringhoffer, M., Arand, M., Karbach, J., Ilsemann, C., Hagedorn, M., and Knuth, A. (1996a). Generation of cytotoxic T-cell responses with synthetic melanoma-associated peptides in vivo: implications for tumor vaccines with melanoma-associated antigens. Int. J. Cancer *66*, 162–169.

Jager, E., Ringhoffer, M., Dienes, H.P., Arand, M., Karbach, J., Jager, D., Ilsemann, C., Hagedorn, M., Oesch, F., and Knuth, A. (1996b). Granulocyte-macrophage-colony-stimulating factor enhances immune responses to melanoma-associated peptides in vivo. Int. J. Cancer *67*, 54–62.

Jager, E., Ringhoffer, M., and Karbach, J. (1996c). Inverse relationship of melanocyte differentiation antigen expression in melanoma tissues and CD8+ cytotoxic-T-cell responses: evidence for immunoselection of antigen-loss variants in vivo. Int. J. Cancer *66*, 470–476.

Jager, E., Chen, Y.T., and Drijfhout, J.W. (1998). Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J. Exp. Med. *187*, 265–270.

Kawakami, Y., Eliyahu, S., Delgado, C.H., Robbins, P.F., Rivoltini, L., Topalian, S.L., Miki, T., and Rosenberg, S.A. (1994a). Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc. Natl. Acad. Sci. USA *91*, 3515–3519.

Kawakami, Y., Eliyahu, S., Delgado, C.H., Robbins, P.F., Sakaguchi, K., Appella, E., Yannelli, J.R., Adema, G.J., Miki, T., and Rosenberg, S.A. (1994b). Identification of a human melanoma antigen recognized by tumor infiltrating lymphocytes associated with in vivo tumor rejection. Proc. Natl. Acad. Sci. USA *91*, 6458–6462.

Kawakami, Y., Eliyahu, S., Sakaguchi, K., Robbins, P. F., Rivoltini, L., Yannelli, J.R., Appella, E., and Rosenberg, S.A. (1994c). Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2 restricted tumor infiltrating lymphocytes. J. Exp. Med. *180*, 347–352.

Kawakami, Y., Eliyahu, S., Jennings, C., Sakaguchi, K., Kang, X., Southwood, S., Robbins, P.F., Sette, A., Appella, E., and Rosenberg, S.A. (1995). Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor infiltrating T-lymphocytes associated with in vivo tumor regression. J. Immunol. *154*, 3461–3968.

Kawakami, Y., Robbins, P.F., Wang, X., Tupesis, J.P., Parkhurst, M.R., Kang, X., Sakaguchi, K., Appella, E., and Rosenberg, S.A. (1998). Identification of new melanoma epitopes on melanosomal proteins recognized by tumor infiltrating T lymphocytes restricted by HLA-A21, -A2, and -A3 alleles. J. Immunol. *161*, 6985–6992.

Kittlesen, D.J., Thompson, L.W., and Gulden, P.H. (1998). Human melanoma patients recognize an HLA-A1-restricted CTL epitope from tyrosinase containing two cysteine residues: implications for tumor vaccine development. J. Immunol. *160*, 2099–2106.

Korinek, V., Barker, N., and Morin, P.J. (1997). Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/-colon carcinoma. Science *275*, 1784–1787.

Lehmann, F., Marchand, M., and Hainaut, P. (1995). Differences in the antigens recognized by cytolytic T cells on two successive metastases of a melanoma patient are consistent with immune selection. Eur. J. Immunol. *25*, 340–347.

Lethe, B., Van der Bruggen, P., Brasseur, F., and Boon, T. (1997). MAGE-1 expression threshold for the lysis of melanoma cell lines by a specific cytotoxic T lymphocyte. Melanoma Res. *2*, S83–S88

Loftus, D.J., Appella, E., and Rivoltini, L. (1996). Identification of epitope mimics recognized by CTL reactive to the melanoma/melanocyte-derived peptide MART-1 27–35. J. Exp. Med. *184*, 647–657.

Malarkannan, S., Afkarian, M., and Shastri, N. (1995). A rare cryptic translation product is presented by Kb major histocompatibility complex class I molecule to alloreactive T cells. J. Exp. Med. *182*, 1739–1750.

Mandruzzato, S., Brasseur, F., and Andry, G. (1997). A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. J. Exp. Med. *186*, 785–793.

Marchand, M., Weynants, P., Rankin, E., Arienti, F., Belli, F., Parmiani, G., Cascinelli, N., Bourlond, A., Vanwijck, R., Humble, Y., et al. (1995). Letter to the editor: tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. Int. J. Cancer *63*, 883–885.

Marincola, F.M., Rivoltini, L., Salgaller, M.L., Player, M., and Rosenberg, S.A. (1996). Differential anti-MART-1/MelanA CTL activity in peripheral blood of HLA-A2 melanoma patients in comparison to healthy donors: evidence for in vivo priming by tumor cells. J. Immunother. *19*, 266–277.

Marshall, J.L., Hawkins, M.J., Tsang, K.Y., Richmond, E., Pedicano, J.E., Zhu, M.Z., and Schlom, J. (1999). Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. J. Clin. Oncol. *17*, 332–337.

Morin, P.J., Sparks, A.B., and Korineck, V. (1999). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science *275*, 1787–1790.

Nestle, F.O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G., and Schadendorf, D. (1998). Vaccination of melanoma patients with peptide or tumor lysate-pulsed dendritic cells. Nat. Med. *4*, 328–332.

Parkhurst, M.R., Fitzgerald, E.B., Southwood, S., Sette, A., Rosenberg, S.A., and Kawakami, Y. (1998). Identification of a shared HLA-A*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). Cancer Res. *58*, 4895–4901.

Quelle, D.E., Zindy, F., Ashmun, R.A., and Sherr, C.J. (1995). Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. Cell *83*, 993–1000.

Robbins, P.F., El-Gamil, M., Kawakami, Y., and Rosenberg, S.A. (1994). Recognition of tyrosinase by tumor infiltrating lymphocytes from a patient responding to immunotherapy. Cancer Res. *54*, 3124–3126.

Robbins, P.F., El-Gamil, M., Li, Y., Topalian, S.L., Rivoltini, L., Sakaguchi, K., Appela, E., Kawakami, Y., and Rosenberg, S.A. (1995). Cloning of a new gene encoding an antigen recognized by melanoma-specific HLA-A24-restricted tumor-infiltrating lymphocytes. J. Immunol. *154*, 5944–5950.

Robbins, P.F., El-Gamil, M., Li, Y.F., Kawakami, Y., Loftus, D., Appella, E., and Rosenberg, S.A. (1996). A mutated B-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J. Exp. Med. *183*, 1185–1192.

Robbins, P.F., El-Gamil, M., and Li, Y.F. (1997). The intronic region of an incompletely spliced gp100 gene transcript encodes an epitope recognized by melanoma-reactive tumor-infiltrating lymphocytes. J. Immunol. *159*, 303–308.

Rosenberg, S.A. (1996). Development of cancer immunotherapies based on identification of the genes encoding cancer regression antigens. J. Natl. Cancer Inst. *88*, 1635–1644.

Rosenberg, S.A. (1997). Cancer vaccines based on the identification of genes encoding cancer regression antigens. Immunol. Today *18*, 175–182.

Rosenberg, S.A., and White, D.E. (1996). Brief report: vitiligo in patients with melanoma: normal tissue antigens can be targets for cancer immunotherapy. J. Immunother. *183*, 81–84.

Rosenberg, S.A., Yang, J.C., Topalian, S.L., Schwartzentruber, D.J., Weber, J.S., Parkinson, D.R., Seipp, C.A., Einhorn, J.H., and White, D.E. (1994). Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin-2. JAMA *271*, 907–913.

Rosenberg, S.A., Yang, J.C., White, D.E., and Steinberg, S.M. (1998a). Durability of complete responses in patients with metastatic cancer treated with high-dose interleukin-2: identification of the antigens mediating response. Ann. Surg. *228*, 307–319.

Rosenberg, S.A., Zhai, Y., Yang, J.C., Schwarzentruber, D.J., Hwu, P., Marincola, F.M., Topalian, S.L., Restifo, N.P., Seipp, C.A., Einhorn, J.H., et al. (1998b). Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. J. Natl. Cancer. Inst. *90*, 1894–1900.

Rosenberg, S.A., Yang, J.C., Schwartzentruber, D.J., Hwu, P., Marincola, F.M., Topalian, S.L., Restifo, N.P., Dudley, M.E., Schwarz, S.L., Spiess, P.J., et al. (1998c). Immunologic and therapeutic evaluation of a synthetic vaccine for the treatment of patients with metastatic melanoma. Nat. Med. *4*, 321–327.

Rubinfeld, B., Robbins, P., and El-Gamil, M. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science *275*, 1790–1792.

Sahin, U., Tureci, O., Schmitt, H., Cochlovius, B., Johannes, T., Schmits, R., Stenner, F., Luo, G., Schobert, I., and Pfreundschuh, M. (1995). Human neoplasms elicit multiple specific immune responses in the autologous host. Proc. Natl. Acad. Sci. USA *92*, 11810–11813.

Schneider, J., Brichard, V., and Boon, T. (1998). Overlapping peptides of melanocyte differentiation antigen Melan-A/MART-1 recognized by autologous cytolytic T lymphocytes in association with HLA-B45.1 and HLA-A2.1. Int. J. Cancer *75*, 451–458.

Sherr, C. J. (1996). Cancer cell cycles. Science *274*, 1672–1677.

Shichijo, S., Nakao, M., and Imai, Y. (1998). A gene encoding antigenic peptides of human squamous cell carcinoma recognized by cytotoxic T lymphocytes. J. Exp. Med. *187*, 277–288.

Sidney, J., Grey, H.M., and Southwood, S. (1996). Definition of an HLA-A3-like super motif demonstrates the overlapping peptide-binding repertoires of common HLA molecules. Hum. Immunol. *45*, 79–93.

Skipper, J.C.A., Hendrickson, R.C., Gulden, P.H., Brichard, V., Van Pel, A., Chen, Y., Shabanowitz, J., Wolfel, T., Slingluff, C.L., Boon, T., et al. (1996a). An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins. J. Exp. Med. *183*, 527–534.

Skipper, J.C., Kittlesen, D.J., Hendrickson, R.C., Deacon, D.D., Harthun, N.L., Wagner, S.N., Hunt, D.F., Engelhard, V.H., and Slingluff, C.L., Jr. (1996b). Shared epitopes for HLA-A3-restricted melanoma-reactive human CTL include a naturally processed epitope from Pmel-17/gp100. J. Immunol. *157*, 5027–5033.

Stockert, E., Jager, E., and Chen, Y.T. (1998). A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J. Exp. Med. *187*, 1349–1354.

Sykulev, Y., Joo, M., Vturina, I., Tsomides, T.J., and Eisen, H.N. (1996). Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. Immunity *4*, 565–571.

Tanaka, F., Fujie, T., and Tahara, K. (1997). Induction of antitumor cytotoxic T lymphocytes with a MAGE-3 encoded synthetic peptide presented by human leukocytes antigen-A24. Cancer Res. 4465–4468.

Topalian, S.L., Rivoltini, L., Mancini, M., Markus, N.R., Robbins, P.F., Kawakami, Y., and Rosenberg, S.A. (1994). Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc. Natl. Acad. Sci. USA *91*, 9461–9465.

Traversari, C., Van der Bruggen, P., Luescher, I.F., Lurquin, C., Chomez, P., Van Pel, A., De Plaen, E., Amar-Costesec, A., and Boon, T. (1992). A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J. Exp. Med. *176*, 1453–1457.

Tsai, V., Southwood, S., and Sidney, J. (1997). Identification of subdominant CTL epitopes of the gp100 melanoma-associated tumor antigen by primary in vitro immunization with peptide-pulsed dendritic cells. J. Immunol. *158*, 1796–1802.

Tsang, K.Y., Zaremba, S., and Nieroda, C.A. (1995). Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. J. Natl. Cancer Inst. *87*, 982–990.

Tureci, O., Sahin, U., Schobert, I., Koslowski, M., Schmitt, H., Schild, H.-J., Stenner, F., Seizt, G., Rammensee, H.-G., and Pfreundschuh, M. (1996). The SSX-2 gene, which is involved in the t(X;18) translocation of synovial sarcomas, codes for the human tumor antigen HOM-MEL-40. Cancer Res. *56*, 4766–4772.

Van Den Eynde, B., Peeters, O., De Backer, O., Gaugler, B., Lucas, S., and Boon, T. (1995). A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J. Exp. Med. *182*, 689–698.

Van Den Eynde, B., and Van der Bruggen, P. (1997). T cell defined tumor antigens. Curr. Opin. Immunol. *9*, 684–693.

Van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De-Plaen, E., Van Den Eynde, B., Knuth, A., and Boon, T. (1991). A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science *254*, 1643–1647.

Van der Bruggen, P., Bastin, J., Gajewski, T., Coulie, G., Boel, P., DeSmet, C., Traversari, C., Townsend, A., and Boon, T. (1994a). A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. Eur. J. Immunol. *24*, 3038–3043.

Van der Bruggen, P., Szikora, J.P., and Boel, P. (1994b). Autologous cytolytic T lymphocytes recognize a MAGE-1 nonpeptide on melanomas expressing HLA-Cw*1601. Eur. J. Immunol. *24*, 2134–2140.

Van Pel, A., Van der Bruggen, P., Coulie, P.G., Brichard, V.G., Lethe, B., Van Den Eynde, B., Uyttenhove, C., Renauld, J.-C., and Boon, T. (1995). Genes coding for tumor antigens recognized by cytolytic T lymphocytes. Immunol. Rev. *145*, 229–250.

Wang, R.-F., Robbins, P.F., Kawakami, Y., Kang, X.Q., and Rosenberg, S.A. (1995). Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. J. Exp. Med. *181*, 799–804.

Wang, R.-F., Parkhurst, M.R., Kawakami, Y., Robbins, P.F., and Rosenberg, S.A. (1996). Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J. Exp. Med. *183*, 1131–1140.

Wang, R.-F., Johnston, S.L., Southwood, S., Sette, A., and Rosenberg, S.A. (1998a). Recognition of an antigenic peptide derived from tyrosinase-related protein-2 by CTL in the context of HLA-A31 and -A33. J. Immunol. *160*, 890–897.

Wang, R.-F., Johnston, S.L., Topalian, S.L., Schwartzentruber, D.J., and Rosenberg, S.A. (1998b). A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. J. Immunol. *161*, 3596–3606.

Weynants, P., Lethe, B., Brasseuer, F., Marchand, M., and Boon, T. (1994). Expression of MAGE genes by non-small-cell lung carcinomas. Int. J. Cancer *56*, 826–829.

Woglom, W.H. (1929). Immunity to transplantable tumors. Cancer Res. *4*, 129.

Wolfel, T., Van Pel, A., Brichard, V., Schneider, J., Seliger, B., Meyer zum Buschenfeld, K.-H., and Boon, T. (1994). Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. Eur. J. Immunol. *24*, 759–764.

Wolfel, T., Hauer, M., Schneider, J., Serrano, M., Wolfel, C., Klehmann-Hieb E., De Plaen, E., Hankeln, T., Meyer Zum Buschenfelde, K.H., and Beach, D. (1995). A p16INK4A-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science *269*, 1281–1284.

総 説

癌ワクチン

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要旨 最近の遺伝子工学や分子生物学の進歩によって多数の癌特異抗原が確認されるようになった。その後，癌特異的免疫療法としてHLA class I 拘束性の細胞傷害性T細胞の役割が多くの研究によって論証された。われわれも，HLA-A 24およびA 26拘束性のCTLを食道扁平上皮癌患者から樹立し，このCTLを用いてcDNA発現クローニング法により新規遺伝子 *SART-1* を同定し，この遺伝子がコードするいくつかの癌拒絶ペプチドを同定した。さらに同様の方法により，HLA-A 24拘束性腫瘍特異的CTLが認識する抗原エピトープをコードする *cyclophilin B* 遺伝子を同定した。現在，われわれはこれらのペプチドワクチンを用いて第I相試験を施行中であるが，*in vitro* の検査で数例にCTL前駆体細胞の増加を認めつつある。しかし最新の研究では，多くの癌細胞はHLA class I 発現を減弱してCTLの認識から逃れていることが報告されるようになり，また癌細胞の大半が免疫抑制物質を産生するという問題がある。それゆえに，癌ワクチン療法を成功させるためには，これらの重要問題を早急に解決しなければならないと考える。

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Specific Immunotherapy with Cancer Vaccines: Hideaki Yamana*1 and Kyogo Itoh*2 (*1*Multidisciplinary Treatment Center, and* *2*Immunology, Kurume University, School of Medicine*)

Summary

With the recent progress in molecular biology and gene technology, many new cancer-specific antigens have been identified. Many studies have demonstrated the role of HLA class I-restricted cytotoxic T lymphocytes (CTLs) in cancer specific-immunotherapies. We have also established HLA-A24-and A26-restricted and cancer-specific CTLs from a patient with squamous cell carcinoma of the esophagus. Using CTLs, we identified a new gene *SART-1* by cDNA-expression cloning and some *SART-1*-derived cancer rejection peptides were also identified. Further more, using the same approach, we identified a *cyclophilin B* gene that encodes antigenic epitopes recognized by HLA-A24-restricted and tumor-specific CTLs. Now we are performing phase I trials using these peptide vaccines and have found an increase in CTL precursor frequency in some cases in an *in vitro* study. However, other recent studies have reported that many tumors escape from CTL recognition by downregulation of HLA class I expression. Moreover, most cancer cells produce a suppressor agents against the immune system. Therefore, we must resolve these major problems to produce successful cancer vaccine therapy soon. Key words: Cancer-specific immunotherapy, Cancer antigen, Cancer vaccine, Address request for reprints to: Dr. Hideaki Yamana, Multidisciplinary Treatment Center, Kurume University, School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan

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はじめに

近年の基礎免疫学の進歩によって，1991年にメラノーマより細胞傷害性T細胞が認識する腫瘍特異抗原[1]の存在が科学的に証明され，癌拒絶抗原の存在と免疫排除機能の概念が確立された。このように，ヒト悪性腫瘍においても動物実験と同様に免疫担当細胞によって拒絶される特異抗原の存在が明らかにされ，癌特異的免疫療法は新たな展開を迎えるに至った。これを契機として多くの施設で癌抗原解析の研究が盛んに行われるようになり，ヒト悪性腫瘍特異抗原が種々同定され，悪性腫瘍の治療を目的として癌特異抗原を用いた癌ワクチン療法の臨床試験が試みられるようになった。

本稿では，癌ワクチンの現況について，われわれが開発した癌ペプチドワクチンを主体に概説し，現時点における問題点と今後の展望について考察する。

I．癌特異的細胞傷害性T細胞（CTL）が認識する腫瘍特異抗原

動物実験ではCTLが主要組織適合性抗原（MHC）拘束性かつ抗原特異的に癌細胞を殺傷することは，周知のとおりであった。しかし，このCTLによる抗原分子の認識機序が明確になったのは十数年前であり，MHC class Iもしくはclass IIドメインによって構築される小溝に8～15個程度のアミノ酸が結合し，これが抗原分子となってT細胞が認識することが判明した。この免疫基礎科学の進歩を基に，1991年にBoonらはヒト・メラノーマ細胞のHLA class I分子上に提示されCTLの標的となる抗原分子をコードする*MAGE*遺伝子[1]を同定した。CTLによって認識される癌拒絶抗原の検索方法はいくつかあるが，Boonらの開発したcDNA-発現クローニング法[1]が最も多くの新しい抗原単離に成功している。また最近では，癌患者の血清中の抗体を用いた簡便な発現クローニング法のSEREX法[2]によっても新たな抗原分子が同定されている。現在までに報告された主なヒト癌拒絶抗原とHLA class I拘束性，ならびにそのペプチドを表1に示す。

1．各種癌および正常精巣に発現する抗原

正常組織では精巣を除いて発現しないものの，各種癌では広範に発現するcancer-testis抗原として，MAGE-1，MAGE-3，BAGE，GAGE-2，RAGE，NA 17-A，NY-ESO-1などがある[3-8]。

2．分化抗原

1）メラノソーム蛋白質

正常のメラノサイトとメラノーマの両者に共通して発現する分化抗原である。MAR T-1/Melan-A，gp 100/Pmel 17，tyrosinase，TRP 2などがある[9-14]。同定されたペプチドの多くはHLA-A 2拘束性であり，正常細胞のメラノサイトにも発現していることから，メラノサイト破壊性自己免疫疾患での標的分子としての可能性が想定される。

3．その他の抗原分子

メラノソーム蛋白以外の分化抗原として，癌細胞表面に発現する分子としては，前立腺癌に発現するprostate-specific membrane antigen（PSMA）やprostate-acid phosphate（PAP）がよく知られている[15-17]。一方，脳に発現するcdr 2抗原が，主要随伴性小脳変性症状（PCD）を来す女性生殖器腫瘍患者から同定され[18]，PCDを伴う悪性腫瘍の病態解明や新治療の開発などに期待がもたれる。

4．腫瘍特異的変異抗原

癌化の過程で起こる遺伝子変化によって作りだされた癌特有の抗原で，変異CDK 4抗原，変異β-カテニン抗原，MUM-1抗原，CASP-8などが知られている[19-23]。また，イントロンが翻訳されるケースとして，変異gp 75がある。これらの抗原は，癌特異的であるが遺伝子変化は個々の腫瘍で異なるため，広く臨床使用するためのワクチン分子とはなりにくい。

5．癌に高頻度で発現する抗原

多くの正常細胞や癌細胞に非変異体としてユビキタスに発現する抗原が，CTLの認識分子となっている場合があり，p 15やHER 2/neu，CEA，MUC-1などがよく知られている[24-27]。

6．扁平上皮癌拒絶抗原

われわれは，食道扁平上皮癌患者より誘導した

表1 ヒト癌拒絶抗原の種類とHLA class I拘束性およびそのペプチド

癌拒絶抗原	HLA拘束性	ペプチド
1．Cancer-Testis (CT) 抗原		
MAGE-1	A 1	EADPTGHSY
	Cw 16	SAYEPRKL
MAGE-3	B 44	MEVDPIGHLY
TRP 2	A 31	LLPGGRPYR
	A 33	LLPGGRPYR
PSA-1	A 2	FLTPKKLQCV
PSA-3	A 2	VISNDVCAQV

現する抗原
しないものの，
er-testis 抗原と
GE，GAGE-2，
などがある[3-8]。

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osinase，TRP 2
チドの多くは
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ラノサイト破壊
ての可能性が想

原として，癌細
前立腺癌に発現
rane antigen
ate (PAP) が
発現する cdr 2
(PCD) を来す
[8]，PCD を伴う
発などに期待が

によって作りだ
4 抗原，変異 β-
SP-8 などが知
ンが翻訳される
これらの抗原
ま個々の腫瘍で
ものワクチン分

単体としてユビ
認識分子となっ
1/neu，CEA，
[-27]。

ましより誘導した

表 1 ヒト癌拒絶抗原の種類と HLA class I 拘束性およびそのペプチド

癌拒絶抗原	HLA 拘束性	ペプチド
1．Cancer-Testis (CT) 抗原		
MAGE-1	A 1	EADPTGHSY
	Cw 16	SAYEPRKL
MAGE-3	B 44	MEVDPIGHLY
	A 1	EVDPIGHLY
BAGE	A 2	FLWGPRALV
GAGE-1，GAGE-2	Cw 16	AARAVFLAL
RAGE	CW 6	YRPRPRRY
NA 17-A	B 7	SPSSNRIRNT
NY-ESO-1	A 2	VLPDVFIRC
	A 2	QLSLLMWIT
	A 2	SLLMWITQC
	A 2	SLLMWITQCFL
2．分化抗原		
MART-1	A 2	AAGIGILTV
	A 2	EAAGIGILTV
	A 2	ILTVILGVL
	B 45	AEEAAGIGIL
	B 45	AEEAAGIGILT
gp 100/Pmel 17	A 2	KTWGQYWQV
	A 2	AMLGTHTMEV
	A 2	MLGTHTMEV
	A 2	ITDQVPFSV
	A 2	YLEPGPVTA
	A 2	LLDGTATLRL
	A 2	VLYRYGSFSV
	A 2	SLADTNSLAV
	A 3	ALLAVGATK
	A 24	VYFFLPDHL
tyrosinase	A 1	DAEKCDICTDEY
	A 2	MLLAVLYCL
	A 2	YMNGTMSQV
	A 24	AFLPWHRLF
	B 44	SEIWRDIDF
	DR 4	QNILLSNAPLGPQFP
	DR 4	SYLQDSDPDSFQD
TRP 2	A 31	LLPGGRPYR
	A 33	LLPGGRPYR
PSA-1	A 2	FLTPKKLQCV
PSA-3	A 2	VISNDVCAQV
PSA-9	A 3	QVHPQKVTK
PAP-1	A 2	LLLARAASL
PAP-5	A 2	ALDVYNGLL
PAP-7	A 2	VLAKELKFV
cdr 2-1	A 2	KLVPDSLYV
cdr 2-2	A 2	SLLEEMFLT
3．癌特異的変異抗原		
変異 CDK 4	A 2	ACDPHSGHFV
変異 β-catenin	A 24	SYLDSGIHF
LB 33 (MUC-1)	B 44	EEKLIVVLF
変異 gp 75 (TRP-1)	A 31	MSLQRQFLR
変異 HLA-A 2	A 2	CVEWLRIYLENGK
CASP-8	B 35	FPSDSWCYF
4．癌に高発現する抗原		
p 15	A 24	AYGLDFYIL
HER 2/neu	A 2	IISAVVGIL
PRAME	A 24	LYVDSLFFL
GnT-V	A 2	VLPDVFIRC
CEA	A 2	
Muc-1	A 11	STAPPHGV
5．扁平上皮癌拒絶抗原		
SART-1	A 24	EYRGFTQDF
	A 26	KGSGKMKTE
	A 26	KLDEEALLK
	A 26	VLSGSGKSM
SART-2	A 24	DYSARWNEI
	A 24	SYTRLFLIL
SART-3	A 24	VYDYNCHVDL
	A 24	AYIDFEMKI

HLA-A 2602拘束性CTLによって認識される癌抗原ペプチドが存在することを報告[28]した。また，食道や肺の扁平上皮癌のみならず肺，胃，大腸，乳腺の腺癌にもHLA-A 24およびHLA-A 2結合性癌抗原ペプチドが存在することを発見し[29-32]，メラノーマ以外にも癌退縮抗原が存在することを証明した。

II. 欧米における癌ワクチンの現況と成績

1. メラノーマ

1995年にベルギーのLugwig癌研究所において，HLA-A 1陽性の転移性メラノーマ患者12例に対するpilot studyとして，MAGE-3ペプチドの単独投与が施行[33]された。この成績をみると，治療完遂例は6例で，このうち3例にpartial response(PR)を認め，残りの3例はprogressive disease (PD) であったと報告し，1999年には完遂25例中7例に腫瘍縮小効果が得られたと報告[34]している。また1998年には，米国癌研究所(NIH)のRosenbergらによって，HLA-A 2陽性転移性メラノーマ患者に対する第I相試験として，gp 100由来ペプチドと不完全フロインド・アジュバント (IFA) の併用投与が施行され，21例中20例の末梢血単核球 (PBMC) にHLA-A 2拘束性メラノーマ特異的CTLが誘導され，*in vivo* 臨床試験において初めてペプチドワクチンの効果が確認された。またさらに，アジュバントとして高用量のIL-2を用いたところ，31例中13例に腫瘍縮小効果を認めたと報告[35]している。しかし，高用量IL-2の単独投与においても16%に明らかな腫瘍縮小効果がみられることが報告[36]され，メラノーマに対するペプチドワクチンの臨床効果の判定のためには今後さらなる検討が必要である。また，同年にはスイスにおいて，メラノーマで同定された数種類のペプチドを患者より採取した樹状細胞 (DC) に *ex vivo* で感作し，それを生体内に移入する細胞療法の第I相試験の成績が報告[37]され，転移性メラノーマ16例中2例にcomplete response (CR) を，3例にPRを認めている。

2. 大腸癌

Eastern Cooperative Oncology Group (ECOG) により，Stage II，IIIの大腸癌患者の術後補助療法として放射線照射自己癌細胞とBCGによるワクチン療法の第III相試験が施行された。外科手術単独群と外科手術＋ワクチン療法群の予後の比較において，無病生存曲線では$p=0.078$，全生存曲線では$p=0.13$とワクチン療法群が良好の傾向を示したと報告[38]している。その他にも腫瘍抗原関連抗体ワクチンを用いた臨床試験がいくつか行われているが，有効性に関して明確な結論は得られていない。

3. 前立腺癌

前立腺癌に対するワクチン療法の主体は，DCにHLA-class I拘束性PSMA特異的ペプチドを感作した細胞療法とGM-CSFの併用，もしくはGM-CSF遺伝子導入放射線照射自己腫瘍細胞によるワクチン療法であるが，これらの臨床効果は未だ一定していない[39-42]。

4. 腎細胞癌

腎細胞癌のワクチン療法の主体は，IL-2やinterferon(IFN)-α，β，γ，およびGM-CSFなどのサイトカインと自己癌細胞ワクチン，もしくはDC療法が主体である[43-47]。このたびドイツで施行された臨床試験の成績[48]をみると，自己癌細胞とDCのハイブリッドワクチンによって，17例中7例にCR/PRの抗腫瘍効果が得られたと報告しており，今後の成果が期待される。

5. その他

膀胱癌や肺癌，子宮癌でもいくつかワクチン療法が施行[49-51]されているが，臨床効果に関しては未だ明確な結論はでていない。

III. 本邦における癌ワクチンの現況

本邦においても1990年代後半に，MAGE-3ペプチドやCEAワクチン[52]，MUC-1ペプチドなどを用いた臨床試験が施行され，その成績の一部は研究会や学会などでも発表されている。また，珠玖らはHLA-A 24陽性腺癌患者を対象として，HER 2/neuペプチドによる臨床試験を開始している。

われわれは，上皮性癌拒絶抗原の同定とその臨床応用を目的として，今までに七つの拒絶抗原とそれらにコードされる十数種類のペプチド抗原を同定しており，SART-1[53-59]，SART-2[60]，SART-

3[61]，SART-4
り，cyclophili
膀胱腺癌細胞
癌患者より樹
CTLが，p 56
し，HLA-A 2
よび10-mer
ペプチドのな
A 24もしくは
平上皮癌に対
A 24陽性大腸
ペプチドはH
Lckペプチド
転移性癌に対
機中である。
そこで，こ
しているSAR
ペプチドのCT
なお，CTL
を表2に示す。
1. SART-
1) *SART-*
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を用いたcDN
KE 4-CTLが
伝子をプロー
PBMCのcDN
えられる2,50
れた。

表 2　久留米大学病院において臨床試験施行中の癌抗原ペプチド

抗原	HLA-A locus	ペプチド
SART-1	HLA-A 26 (01, 02, 03)	KGSGKMKTE
	HLA-A 2402	EYRGFTQDF
SART-3	HLA-A 2402	VYDYNCHVDL
		AYIDFEMKI
CypB	HLA-A 2402	KFHRVIKDF
		KYHRVIKDF（改変ペプチド）
		DFMIQGGDF
		DYMIQGGDF（改変ペプチド）

3[61]，SART-4は食道扁平上皮癌細胞のcDNAより，cyclophilin B(CypB)[62]，ART-1，ART-4は膀胱腺癌細胞のcDNAより同定した。また，食道癌患者より樹立したHLA-A 24拘束性癌特異的CTLが，p 56[lck] (Lck)[63]を認識することを見いだし，HLA-A 24結合モチーフを有する9-merおよび10-merのLckペプチドを合成した。これらペプチドのなかで，SART-1ペプチドはHLA-A 24もしくはHLA-A 26陽性の食道および肺扁平上皮癌に対して，SART-3ペプチドはHLA-A 24陽性大腸癌および乳癌に対して，また，CypBペプチドはHLA-A 24陽性肺癌に対して，さらにLckペプチドはHLA-A 24陽性かつLck陽性の転移性癌に対して第Ⅰ相試験を実施，もしくは待機中である。

そこで，これらのなかですでに臨床試験を実施しているSART-1，SART-3，CypBの概要と各ペプチドのCTL誘導能について紹介する。

なお，CTLを誘導するこれらの癌抗原ペプチドを表2に示す。

1．SART-1

1）*SART-1*遺伝子[53]

食道扁平上皮癌患者（HLA-A 2402/A 2601）のPBMCから，自己癌細胞株（KE 4）を抗原としてCTL(KE 4-CTL)が誘導された。このKE 4-CTLを用いたcDNA-発現クローニング法によってKE 4-CTLがコードする遺伝子が得られ，この遺伝子をプローブとしてKE 4細胞株および健常人PBMCのcDNAライブラリーよりほぼ全長と考えられる2,506 bpの*SART-1*遺伝子が同定された。

2）*SART-1*抗原

*SART-1*mRNAは，ほとんどすべての健常組織に発現していたが，KE 4-CTLは自己非癌細胞を認識できなかった。そこで，実際の蛋白レベルでの発現状態を，SART-1融合蛋白を抗原として作製したポリクローナル抗体を用いてウェスタンブロット法で解析してみた。125 kDaのSART-1蛋白は，検索したすべての癌細胞株のみならず増殖活性を有する正常細胞株の核分画にも発現を認めたが，精巣と胎児肝を除く健常組織には検出できなかった（表3）。

一方，最初にクローニングした990 bpの遺伝子は，259アミノ酸からなるもう一つの蛋白をコードしていると考えられた。そこで，ポリクローナル抗体を作製したところ，この抗体は43 kDaの細胞質内分画の蛋白を認識し，ヒト上皮癌細胞株では頭頸部癌，食道扁平上皮癌，肺扁平上皮癌の大半に，また多くの扁平上皮癌組織に発現を認めた。しかし，増殖性正常細胞やヒト健常組織では，精巣と胎児肝を除いて検出されず，この43 kDaの細胞質内蛋白が癌拒絶抗原と想定された。

3）*SART-1*ペプチド

a．HLA-A 26結合性SART-1ペプチド

SART-1分子内において，HLA-A 2601分子と結合モチーフを有する22種類のオリゴペプチドを合成した。*HLA-A 2601*遺伝子を導入したVA 13細胞をこれらのペプチドで感作し，KE 4-CTLによる認識状態をIFN-γ産生量と^{51}Cr遊離反応による細胞傷害活性試験で検討した結果，3種類の10アミノ酸（10-mer）よりなるペプチドにおいて有意にIFN-γ産生が誘導され，細胞傷

表 3 正常および腫瘍細胞・組織における SART 蛋白の発現（ウェスタン・ブロット解析による）

		SART-1				SART-2		SART-3			
		細胞株		組織		細胞株	組織	細胞株		組織	
		細胞質	核	細胞質	核			細胞質	核	細胞質	核
正常細胞・組織	PBMC	0/5	0/5			0/3		0/5	0/5		
	芽球化 PBMC	0/2	2/2					3/3	0/3		
	線維芽細胞	0/2	2/2			0/1		2/2	0/2		
	胎児肝臓			1/1	1/1		0/1			1/1	0/1
	新生児肝臓			0/1	0/1		0/1			0/1	0/1
	肝臓			0/1	0/1		0/1			0/10	0/10
	精巣			3/3	1/1		0/2			2/2	2/2
	胎盤			0/2	0/1		0/1			0/1	0/1
	食道			0/4	0/2		0/1			0/3	0/3
	膵臓			0/1	0/1		0/1				
	肺						0/1				
	腎臓						0/1				
	胸腺						0/1				
	卵巣						0/1				
癌細胞・組織	頭頸部扁平上皮癌	3/5	2/2	7/7	2/2	8/8	15/16	2/2	2/2	14/20	10/20
	食道扁平上皮癌	4/6	5/5	18/30	3/5	5/5	5/9	8/8	8/8	3/5	4/5
	肺扁平上皮癌	3/3	2/2	8/17	3/4	3/6	4/14	4/4	4/4	7/10	6/10
	肺腺癌	3/6	3/3	16/35	7/7	2/2	6/10	3/3	3/3	5/8	4/8
	乳癌					0/3	0/16	12/12	12/12	4/4	4/4
	メラノーマ	0/2	1/1	0/10		1/1	3/8	2/2	2/2	8/9	8/9
	白血病	0/16	4/4	0/10	4/4	15/15	3/4				

陽性例数/検体数

害活性では SART-$1_{736-745}$ が最も高い値を示した。また，これらの 10-mer 内の 9-mer ペプチド部分（KGSGKMKTE）が KE 4-CTL によって認識されることが判明し，KE 4 細胞株に対する特異的 CTL 誘導能を検討した。その結果，SART-$1_{736-744}$ ペプチドで誘導された PBMC およびそのサブラインのみが特異的かつ高い細胞傷害活性を示し，臨床応用可能と判定し，臨床試験を実施することとなった（図 1）。

b．HLA-A 24 結合性 SART-1 ペプチド

KE 4-CTL はもう一つの HLA class I アレール A 2402 があり，HLA-A 2601 拘束性 CTL 株と同様の手法によって HLA-A 2402 拘束性の CTL 株が樹立された。この CTL 株を用いた解析により，125 kDa および 43 kDa の両蛋白に共通する 3′側から 62 アミノ酸部分に 1 か所 HLA-A 2402 強結合性のペプチド抗原[5]が存在した。そこで，KE 4-CTL 株によって認識されるペプチドを，HLA-A 2402 結合性モチーフをもつ 12 種類の異なる SART-1 蛋白由来ペプチドを用いて検討したところ，オリジナルペプチドの SART-$1_{690-698}$（EYRGFTQDF）が最も強く認識された。このペプチドで感作すると，癌患者のみならず健常人の PBMC より HLA-A 24 拘束性癌特異的 CTL が誘導されることが判明し，臨床応用の可能性が示され，臨床試験を実施することとした（図 1）。

2．*SART-3* 遺伝子

1）*SART-3* 遺伝子

SART-3 遺伝子[60]は，2.9 kbp の ORF の塩基配列を有し，963 個のアミノ酸からなる蛋白をコードしていた。

2）SART-3 抗原

SART-3 の mRNA レベルでの発現をみると，癌のみならずすべての健常組織でも発現を認め

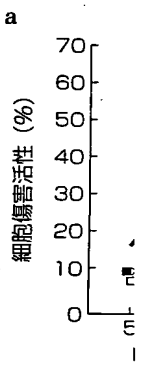
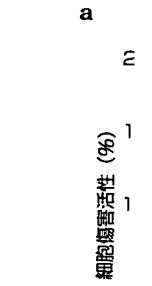


図 1



た。そこで SA
ノ酸からなる
体を作製した。
正常組織では
児肝（細胞質
株ならびに癌
株の細胞質と
織にも細胞質

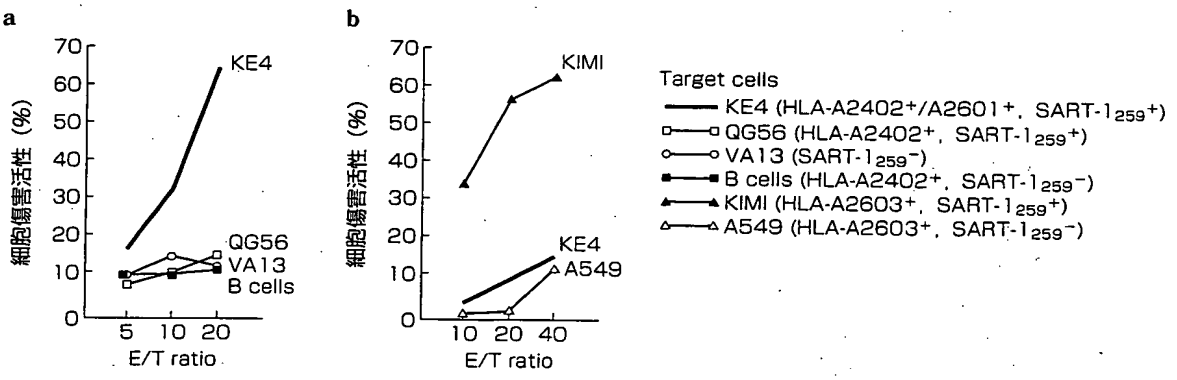


図 1　末梢血リンパ球から SART-1 ペプチドで誘導した HLA class Ⅰ拘束性癌特異的 CTL の細胞傷害活性（^{51}Cr 遊離反応による）
a: HLA-A 24 拘束性 SART-$1_{690-698}$，b: HLA-A 26 拘束性 SART-$1_{736-744}$

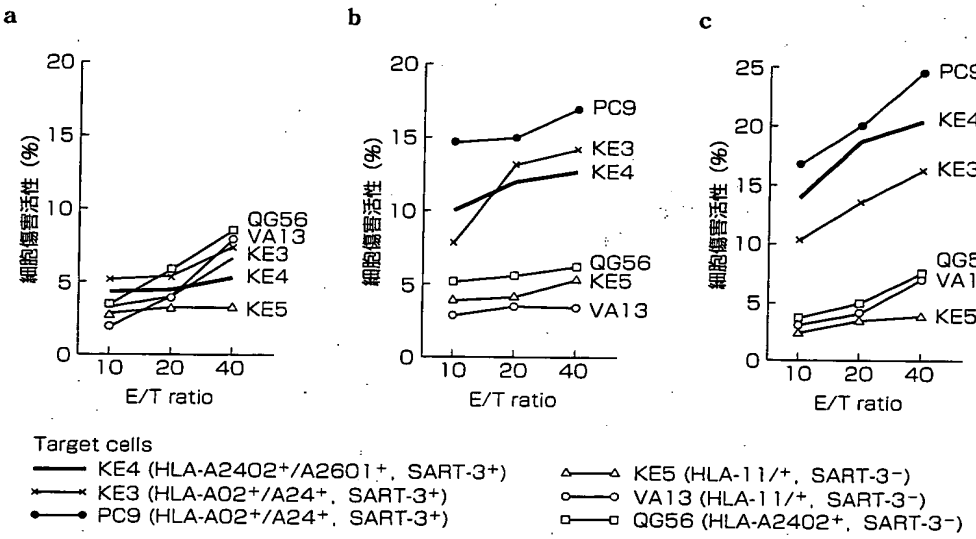


図 2　末梢血リンパ球から SART-3 ペプチドで誘導した HLA-A 24 拘束性癌特異的 CTL の細胞傷害活性（^{51}Cr 遊離反応による）
a: ペプチド刺激なし，b: SART-$3_{109-118}$，c: SART-$3_{315-323}$

た。そこで SART-3 融合蛋白を作製し，963 アミノ酸からなる SART-3 抗原でモノクローナル抗体を作製した。140 kDa の SART-3 分子は，ヒト正常組織では精巣（細胞質および核内分画）と胎児肝（細胞質分画）のみに発現を認めた。癌細胞株ならびに癌組織では，検索したすべての癌細胞株の細胞質と核内に発現を認め，また大半の癌組織にも細胞質および核内に発現を認めた（表 1）。

3）SART-3 ペプチド

SART-3 分子内において，HLA-A 2402 分子と結合モチーフを有する 21 種類の 8-mer～10-mer のオリゴペプチドを合成し，*HLA-A2402* 遺伝子を導入した VA 13 細胞をこれらのペプチドで感作し，KE 4-CTL による認識状態を検討した。その結果，3 種類のペプチドで CTL が誘導され，そのうち 2 種類（VYDYNCHVDL, AYIDFEM KI）が有意に高い IFN-γ 産生を誘導した。また，これ

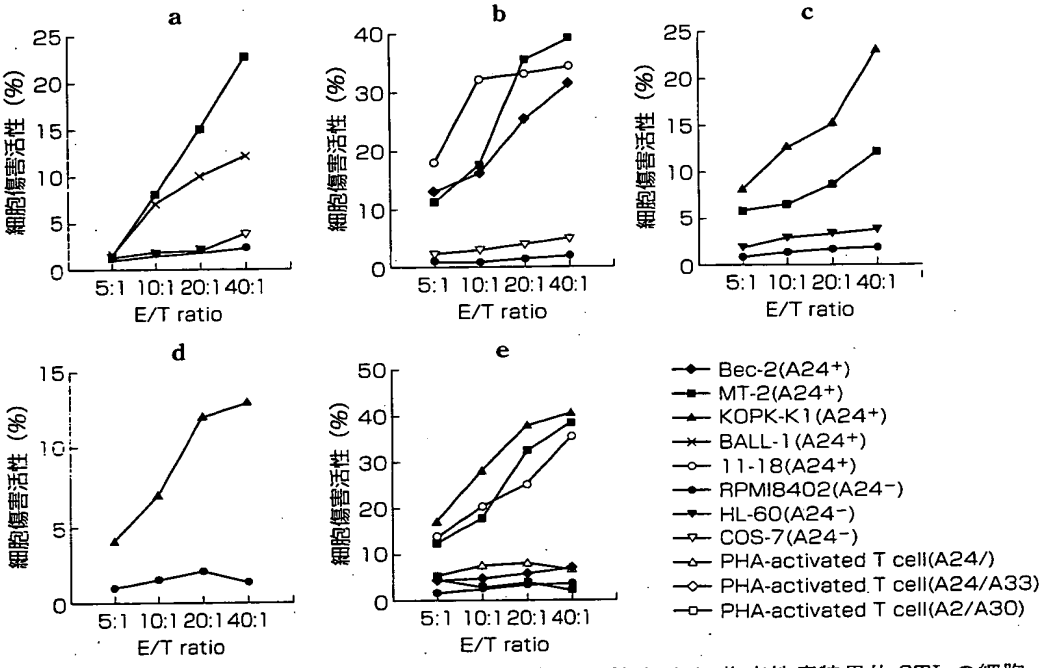


図 3 末梢血リンパ球から CypB ペプチドで誘導した HLA-A 24 拘束性癌特異的 CTL の細胞傷害活性（^{51}Cr 遊離反応による）

a: $CypB_{84-92}$で 3 回刺激した白血病患者の CTL 活性，b: C$CypB_{84-92}$改変ペプチドで 3 回刺激した白血病患者の CTL 活性，c: $CypB_{84-92}$改変ペプチドで 3 回刺激した胃癌患者の CTL 活性，d: $CypB_{84-92}$ 改変ペプチドで 3 回刺激した健常人の CTL 活性，e: $CypB_{91-97}$改変ペプチドで 3 回刺激した健常人の CTL 活性

らのペプチドは，HLA-A 24 陽性癌患者の PBMC からのみ CTL を誘導し，健常人 PBMC からは誘導できなかった。

以上の結果から，SART-3 ペプチドは HLA-A 24 陽性の上皮性癌患者すべてに対する癌ワクチン分子として臨床応用可能と考えられ，臨床試験を開始することとした（図 2）。

3．Cyclophilin B（CypB）

1）*CypB* 遺伝子[61]

肺癌局所に浸潤する T 細胞より HLA-A 24 拘束性 CTL 株（GK-CTL）を樹立し，この CTL によって認識される抗原ペプチドをコードする遺伝子を，cDNA-発現クローニング法で同定した。なお，GK-CTL 株の CTL 活性は，HLA-A 24 拘束性かつ癌細胞特異的であり，HLA-A 24 陽性の芽球化正常リンパ球に対して細胞傷害活性を示さなかった。また，これらの CTL 活性は抗 CD 8 や抗 HLA class I 抗体によって抑制された。IFN-γ 産生量による感受性試験により，GK-CTL は HLA-A 24 陽性の膀胱癌細胞株（HT 1376）に最も高い感受性を示したことから，HT 1376 細胞から 10^5クローンからなる cDNA ライブラリーを作製し，これらのクローンのうちで GK-CTL 株に認識される抗原ペプチドをコードする遺伝子をクローニングしたこの遺伝子配列を解析した結果，*CypB* 遺伝子とまったく同一であった。

2）CypB 抗原

ノーザンブロット法により，各種細胞株における CypB mRNA 発現を検討した結果，すべての細胞に発現がみられたが，特に HT 1376 細胞に顕著であった。また，これらの細胞株における HLA-A 24 発現強度と GK-CTL 株との反応性を IFN-γ 産生量でみたところ，癌細胞中では HT 1376 細胞が最も強い反応を示した。しかし，

CypB陽性でHLA-A 24発現のみられないLK 79細胞株ではIFN-γ誘導能を認めなかった。

3）CypBペプチド

CypB分子内で，HLA-A 2402分子と結合モチーフを有する13種のオリゴペプチド（8-mer～10-mer）を合成し，*HLA-A 2402*遺伝子導入COS 7細胞株をこれらペプチドで感作し，GK-CTL株による認識程度をIFN-γ産生量で検討した。その結果，3種類のペプチドが反応を示し，なかでも2種類（KFHRVIKD，DFMIQGGDF）が用量依存性に特異的な反応を示した。そこで，これら2種類のペプチドについてCTL誘導能を検討した。両ペプチドを*in vitro*で3～4回感作することによって，HLA-A 24陽性白血病患者5例すべてのPBMCから腫瘍特異的CTLの誘導が可能であった。しかし，胃癌や肺癌などの上皮性癌患者，および健常人のPBMCからはCTL誘導が得られなかった。そこで，両ペプチド分子のN'末端から2番目のアミノ酸のフェニルアラニン（F）をタイロシン（Y）に置換した改変ペプチドを合成し，それらのCTL誘導能を検討した。その結果，両改変ペプチドは上皮性癌患者のみならず健常人のPBMCからCTL誘導活性を有した。また，誘導されたCTLは，^{51}Cr遊離法による細胞傷害活性試験で肺癌細胞株や白血病細胞株に有意に強い殺細胞効果を示す反面，芽球化正常リンパ球にはまったく細胞傷害活性を示さず，上皮性癌患者に対する癌ワクチンとして臨床応用可能と判断され，臨床試験を開始した（図3）。

IV. 癌ワクチン療法の問題点と今後の展望

現時点における癌ワクチン療法は，未だ試験中の段階であり，明らかな有効性を論じるまでには至っていない。しかし，前述の臨床試験の成績からは顕著な腫瘍縮小効果を期待することは難しく，さらなる研究が必要である。癌ペプチドワクチン療法の現時点の重要な問題は，CTL前駆細胞は増加してもその細胞には殺細胞効果は弱く，これら誘導増殖した前駆細胞をいかに活性化するかが必要になる。また，生体内でCTLが増加しても，その細胞数には限度があり，CTLのみで癌腫を消滅することは不可能と考えられる。画像診断上，明らかな抗腫瘍効果を得るためには，NK細胞やLAK細胞などの抗腫瘍活性を有するすべての免疫細胞を動員する必要があり，またDCやCD 4陽性細胞などとの相互関係が重要になる。さらに，腫瘍自体が有する免疫エスケープ機構や免疫抑制物質産生などについてもさらなる研究によって解明してゆかねばならず，癌ワクチン療法の臨床効果のみを早急に求めることなく，上記の問題に対して基礎研究と臨床研究の両面から一歩ずつ解決することが癌ワクチン療法確立の近道と思う。

おわりに

癌拒絶抗原MAGE-1が同定されてから10年が経過したが，ヒト癌拒絶抗原を標的とした治療は未だ臨床試験の段階であり，得られたデータを基に今後さらに慎重に研究を重ねてゆかねばならない。現時点の癌特異的免疫療法として最も効果的な方法は，患者のリンパ球をIL-2存在下で自己癌細胞を抗原として活性化し，これを直接癌局所に注入するadoptive immunotherapy[64]と考える。しかし，本治療は患者個々に特異的な治療法であり，全世界に約800万人以上の癌患者が存在する現状からみると，決して有意義な治療法とはいえない。これらの多数の癌患者に，有効な癌特異的免疫療法を行うためには，是非とも有効性の高い多くの癌ワクチンを開発し，臨床試験で実証してゆかなければならない。

文献

1) van der Bruggen P, Traversari C, Chomez P, *et al*: A gene encoding an antigen recognized by cytotoxic T lymphocytes on a human melanoma. *Science* **254**: 1643-1647, 1991.
2) Sahin U, Tureci O, Schmitt H, *et al*: Human neoplasm elicit multiple specific immune responses in the autologous host. *Proc Natl Acad Sci U S A* **92**: 11810-11813, 1995.
3) Traversari C, van der Bruggen P, Luescher IF, *et al*: A nonapeptide encoded by human gene MAGE-1 is restricted on HLA-A 1 by cytolytic T lymphocytes directed against tumor antigen MZ 2-E. *J Exp Med* **176**: 1453-1457, 1992.
4) Guilloux Y, Lucas S, Brichard VG, *et al*: A peptide recognized by human cytotoxic T

lymphocytes on HLA-A 2 melanoma is encoded by an intron sequence of the N-acetylglucosaminyl transferase V gene. *J Exp Med* **183**: 1173-1183, 1996.
5) Gaugler B, Van den Eynde B, van der Bruggen P, *et al*: Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytotoxic T lymphocytes. *J Exp Med* **179**: 921-930, 1994.
6) Boel P, Wildmann C, Sensi ML, *et al*: BAGE: A new gene encoding an antigen recognized on human melanoma by cytotoxic T lymphocytes. *Immunity* **2**: 167-175, 1995.
7) Van den Eynde B, Peeters O, Backer O, *et al*: A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J Exp Med* **182**: 689-698, 1995.
8) Jager E, Chen YT, Drijfhout JW, *et al*: Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO 1-1: Definition of human histocompatibility leukocyte antigen (HLA)-A 2-binding peptide peitope. *J Exp Med* **187**: 265-270, 1998.
9) Kawakami Y, Eliyahu S, Delgado CH, *et al*: Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc Natl Acad Sci U S A* **91**: 3515-3519, 1994.
10) Kawakami Y, Eliyahu S, Delgado CH, *et al*: Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with *in vivo* tumor rejection. *Proc Natl Acad Sci U S A* **91**: 6458-6462, 1994.
11) Brichard V, Van Pel A, Wolfel T, *et al*: The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A 2 melanomas. *J Exp Med* **178**: 489-495, 1993.
12) Lupetti R, Pisarra P, Verrecchia A, *et al*: Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. *J Exp Med* **188**: 1005-1016, 1998.
13) Coulie PG, Brichard V, Van Pel A, *et al*: A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A 2 melanomas. *J Exp Med* **180**: 35-42, 1994.
14) Wang RF, Parkhurst MR, Kawakami Y, *et al*: Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. *J Exp Med* **183**: 1131-1140, 1996.
15) Correale P, Walmsley K, Zaremba S, *et al*: Generation of human cytolytic T lymphocyte lines directed against prostate-specific antigen (PSA) employing a PSA oligoepitope peptide. *J Immunol* **161**: 3186-3194, 1998.
16) Peshwa MV, Shi JD, Ruegg C, *et al*: Induction of prostate tumor-specific CD 8+ cytotoxic T-lymphocytes *in vitro* using antigen-presenting cells pulsed with prostatic acid phosphatase peptide. *Prostate* **36**: 129-138, 1998.
17) Fong L, Ruegg CL, Brockstedt D, *et al*: Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization: implications for immunotherapy of prostate cancer. *J Immunol* **159**: 3113-3117, 1997.
18) Albert ML, Darnell JC, Bender A, *et al*: Tumor-specific killer cells in paraneoplastic cerebellar degeneration. *Nat Med* **4**: 1321-1324, 1998.
19) Robbins PF, El-Gamil M, Li YF, *et al*: A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J Exp Med* **183**: 1185-1192, 1996.
20) Wolfel T, Hauer M, Schneider J, *et al*: A p 16 INK 4 a-insensitive CDK 4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* **269**: 1281-1284, 1995.
21) Coulie PG, Lehmann F, Lethe B, *et al*: A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. *Proc Natl Acad Sci U S A* **92**: 7976-7980, 1995.
22) Brandle D, Brasseur F, Weynants P, *et al*: A mutated HLA-A 2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma. *J Exp Med* **183**: 2501-2508, 1996.
23) Mandruzzato S, Brasseur F, Andry G, *et al*: A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. *J Exp Med* **186**: 785-793, 1996.
24) Robbins PF, El-Gamil M, Li YF, *et al*: Cloning of a new gene encoding an antigen recognized by melanoma-specific HLA-A 24-restricted tumor-infiltrating lymphocytes. *J Immunol* **154**: 5944-5950, 1995.
25) Peoples GE, Goedegebuure PS, Smith R, *et*

al: Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER 2/neu-derived peptide. *Proc Natl Acad Sci U S A* **92**: 432-436, 1995.
26) Yoshino I, Goedegebuure PS, Peoples GE, *et al*: HER 2/neu-derived peptides are shared antigens among human non-small cell lung cancer and ovarian cancer. *Cancer Res* **54**: 3387-3390, 1994.
27) Ioannides CG, Fisk B, Jerome KR, *et al*: Cytotoxic T cells from ovarian malignant tumors can recognize polymorphic epithelial mucin core peptides. *J Immunol* **151**: 3693-3703, 1993.
28) Nakao M, Yamana H, Imai Y, *et al*: HLA A 2601-restricted CTLs recognize a peptide antigen expressed on squamous cell carcinoma. *Cancer Res* **55**: 4248-4252, 1995.
29) Seki N, Hoshino T, Kikuchi M, *et al*: HLA-A locus-restricted and tumor-specific CTLs in tumor-infiltrating lymphocytes of patients with non-small cell lung cancer. *Cell Immunol* **175**: 101-110, 1997.
30) Gohara R, Nakao M, Ogata Y, *et al*: Histocompatibility leukocyte antigen-A 2402-restricted cytotoxic T lymphocytes recognizing adenocarcinomas in tumor-infiltrating lymphocytes of patients with colon cancer. *Jpn J Cancer Res* **88**: 198-204, 1997.
31) Hoshino T, Seki N, Kikuchi M, *et al*: HLA class- I -restricted and tumor-specific CTL in tumor-infiltrating lymphocytes of patients with gastric cancer. *Int J Cancer* **70**: 631-638, 1997.
32) Toh U, Yamana H, Nakao M, *et al*: HLA class I -restricted and tumor-specific cytotoxic T lymphocytes from metastatic lymph nodes of esophageal cancers. *Cell Immunol* **177**: 137-143, 1977.
33) Marchand M, Weynants P, Rankin E, *et al*: Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. *Int J Cancer* **63**: 883-885, 1995.
34) Marchand M, van Baren N, Weynants O, *et al*: Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A 1. *Int J Cancer* **80**: 219-230, 1999.
35) Rosenberg SA, Yang JC, Schwartzentruber DJ, *et al*: Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med* **4**: 321-327, 1998.
36) Atkins MB, Kunkel L, Sznol M, *et al*: High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. *Cancer J Sci Am* **6** (*suppl 1*): S 11-14, 2000.
37) Nestle FO, Alijagic S, Gilliet M, *et al*: Vaccination of melanoma patients with peptide-or tumor lysate-pulsed dendritic cells. *Nat Med* **4**: 328-332, 1998.
38) Harris JE, Ryan L, Hoover HC Jr, *et al*: Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E 5283. *J Clin Oncol* **18**: 148-157, 2000.
39) Simons JW, Mikhak B, Chang JF, *et al*: Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte macrophage colony-stimulating factor using ex vivo gene transfer. *Cancer Res* **59**: 5160-5168, 1999.
40) Slovin SF and Scher HI: Peptide and carbohydrate vaccines in relapsed prostate cancer: immunogenicity of synthetic vaccines in man-clinical trials at Memorial Sloan-Kettering Cancer Center. *Semin Oncol* **26**: 448-454, 1999.
41) Tjoa BA, Simmons SJ, Elgamal A, *et al*: Follow-up evaluation of a phase II prostate cancer vaccine trial. *Prostate* **40**: 125-129, 1999.
42) Simmons SJ, Tjoa BA, Rogers M, *et al*: GM-CSF as a systemic adjuvant in a phase II prostate cancer vaccine trial. *Prostate* **39**: 291-297, 1999.
43) Veelken H, Mackensen A, Lahn M, *et al*: A phase- I clinical study of autologous tumor cells plus interleukin-2-gene-transfected allogeneic fibroblasts as a vaccine in patients with cancer. *Int J Cancer* **70**: 269-277, 1997.
44) Simons JW, Jaffee EM, Weber CE, *et al*: Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by *ex vivo* granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Res* **57**: 1537-1546, 1997.
45) Plautz GE, Bukowski RM, Novick AC, *et al*: T-cell adoptive immunotherapy of metastatic renal cell carcinoma. *Urology* **54**: 617-623, 1999.
46) Schwaab T, Heaney JA, Schned AR, *et al*: A randomized phase II trial comparing two different sequence combinations of au-

tologous vaccine and human recombinant interferon gamma and human recombinant interferon alpha 2 B therapy in patients with metastatic renal cell carcinoma: clinical outcome and analysis of immunological parameters. *J Urol* **163**: 1322-1327, 2000.

47) Rieser C, Ramoner R, Holtl L, *et al*: Mature dendritic cells induce T-helper type-1-dominant immune responses in patients with metastatic renal cell carcinoma. *Urol Int* **63**: 151-159, 2000.

48) Kugler A, Stuhler G, Walden P, *et al*: Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. *Nat Med* **6**: 332-336, 2000.

49) Witjes WP, Konig M, Boeminghaus FP, *et al*: Results of a European comparative randomized study comparing oral bropirimine versus intravesical BCG treatment in BCG-native patients with carcinoma *in situ* of the urinary bladder. European Bropirimine Study Group. *Eur Urol* **36**: 576-581, 1999.

50) Grant SC, Kris MG, Houghton AN, *et al*: Long survival of patients with small cell lung cancer after adjuvant treatment with the anti-idiotypic antibody BEC 2 plus Bacillus Calmette-Guerin. *Clin Cancer Res* **5**: 1319-1323, 1999.

51) van Driel WJ, Ressing ME, Kenter GG, *et al*: Vaccination with HPV 16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial. *Eur J Cancer* **35**: 946-952, 1999.

52) Tsunoda T, Tanimura H, Yamaue H, *et al*: Tumor specific CTL therapy for advanced cancer and development for cancer vaccine. *Hepatogastroenterology* **46** (*suppl 1*): 1287-1292, 1999.

53) Shichijo S, Nakao M, Imai Y, *et al*: A gene encoding antigenic peptides of human squamous cell carcinoma recognized by cytotoxic T lymphocytes. *J Exp Med* **187**: 277-288, 1998.

54) Gotoh M, Shichijo S, Hoshino T, *et al*: Sequence analysis of genes encoding rodent homologues of the human tumor-rejection antigen SART-1. *Jpn J Cancer Res* **89**: 849-854, 1998.

55) Kawamoto M, Shichijo S, Imai Y, *et al*: Expression of the SART-1 tumor rejection antigen in breast cancer. *Int J Cancer* **80**: 64-67, 1999.

56) Matsumoto H, Shichijo S, Kawano K, *et al*: Expression of the SART-1 antigens in uterine cancers. *Jpn J Cancer Res* **89**: 1292-1295, 1998.

57) Kikuchi M, Nakao M, Inoue Y, *et al*: Identification of a SART-1-derived peptide capable of inducing HLA-A 24-restricted and tumor-specific cytotoxic T lymphocytes. *Int J Cancer* **181**: 459-466, 1999.

58) Yamada A, Kawano K, Harashima N, *et al*: Study of HLA class I restriction and the directed antigens of cytotoxic T lymphocytes at the tumor sites of ovarian cancer. *Cancer Immunol Immunother* **48**: 147-152, 1999.

59) Inoue Y, Nakao M, Matsunaga K, *et al*: Induction of HLA-A 26-restricted and tumor specific cytotoxic T lymphocytes by a single peptide of the SART-1 antigen in patient with cancer with different A 26 subtypes. *J Immunother* 2000 (in press).

60) Nakao M, Shichijo S, Imaizumi T, *et al*: Identification of a gene coding for a new squamous cell carcinoma antigen recognized by the CTL. *J Immunol* **164**: 2565-2574, 2000.

61) Yang D, Nakao M, Shichijo S, *et al*: Identification of gene coding for a protein prossessing shared tumor epitopes capable of inducing HLA-A 24-restricted cytotoxic T lymphocytes in cancer patients. *Cancer Res* **59**: 4056-4063, 1999.

62) Gomi S, Nakao M, Niiya F, *et al*: A cyclophilin B gene encodes antigenic epitopes recognized by HLA-A 24-restricted and tumor-specific CTLs. *J Immunol* **163**: 4994-5004, 1999.

63) Veillette A, Foss FM, Sausville EA, *et al*: Expression of the lck tyrosine kinase gene in human colon carcinoma and other non-lymphoid human tumor cell lines. *Oncogene Res* **1**: 357-374, 1987.

64) 山名秀明, 唐　宇飛, 伊東恭悟: 進行食道癌に対する免疫療法. 消化器外科 **23**: 1029-1036, 2000.